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THE Committee appointed by the *Royal Society* to direct the publication of the *Philosophical Transactions* take this opportunity to acquaint the public that it fully appears, as well from the Council-books and Journals of the Society as from repeated declarations which have been made in several former *Transactions*, that the printing of them was always, from time to time, the single act of the respective Secretaries till the Forty-seventh Volume; the Society, as a Body, never interesting themselves any further in their publication than by occasionally recommending the revival of them to some of their Secretaries, when, from the particular circumstances of their affairs, the *Transactions* had happened for any length of time to be intermitted. And this seems principally to have been done with a view to satisfy the public that their usual meetings were then continued, for the improvement of knowledge and benefit of mankind: the great ends of their first institution by the Royal Charters, and which they have ever since steadily pursued.

But the Society being of late years greatly enlarged, and their communications more numerous, it was thought advisable that a Committee of their members should be appointed to reconsider the papers read before them, and select out of them such as they should judge most proper for publication in the future *Transactions*; which was accordingly done upon the 26th of March, 1752. And the grounds of their choice are, and will continue to be, the importance and singularity of the subjects, or the advantageous manner of treating them: without pretending to answer for the certainty of the facts, or propriety of the reasonings contained in the several papers so published, which must still rest on the credit or judgment of their respective authors.

It is likewise necessary on this occasion to remark, that it is an established rule of the Society, to which they will always adhere, never to give their opinion, as a Body,

upon any subject, either of Nature or Art, that comes before them. And therefore the thanks, which are frequently proposed from the Chair, to be given to the authors of such papers as are read at their accustomed meetings, or to the persons through whose hands they received them, are to be considered in no other light than as a matter of civility, in return for the respect shown to the Society by those communications. The like also is to be said with regard to the several projects, inventions, and curiosities of various kinds, which are often exhibited to the Society; the authors whereof, or those who exhibit them, frequently take the liberty to report, and even to certify in the public newspapers, that they have met with the highest applause and approbation. And therefore it is hoped that no regard will hereafter be paid to such reports and public notices; which in some instances have been too lightly credited, to the dishonour of the Society.

PHILOSOPHICAL TRANSACTIONS.

I.—*The Respiratory Exchange of Man During and After Muscular Exercise.**

By J. M. H. CAMPBELL, *O.B.E., B.M.*, C. GORDON DOUGLAS, *C.M.G., M.C., D.M., Fellow of St. John's College, Oxford, and* F. G. HOBSON, *D.S.O., B.M.*

Communicated by Dr. J. S. HALDANE, *F.R.S.*

(Received May 23,—Read December 11, 1919.)

(From the Physiological Laboratory, Oxford.)

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INTRODUCTION.

A good deal of attention has been directed of late to the alteration of the respiratory exchange and respiratory quotient during muscular exercise, with a view to elucidating the character of the metabolism and the behaviour of the respiratory centre. Hitherto, it has, as a rule, been the custom to make only a few determinations of the total respiratory exchange in any one experiment at rather long intervals from one another. Such a method, though it may give the general and broader features of the respiratory exchange, especially when experiments are multiplied, is clearly ill adapted to show any rapid variations that may occur. The individual periods during which the respiratory exchange is actually determined may be too long (this length is often essential, in order to render negligible slight errors which would become significant if it were curtailed), and the long intervals between the different determinations are undesirable.

One of us, in conjunction with HALDANE, HENDERSON, and SCHNEIDER,† attempted to obtain information on the course of the total respiratory exchange in the period of rest immediately following a short and violent muscular exertion at an altitude of

* The experiments described in this paper were made in 1913, and the method we adopted, and some of our results, were described at the Nineteenth International Physiological Congress at Groningen in that year. Pressure of other work prevented us from publishing our results at that time, and during the war it was impossible for us to deal with our material, as we were on military service.

† DOUGLAS, HALDANE, YANDELL HENDERSON, and SCHNEIDER, 'Phil. Trans.,' B, vol. 203, p. 185 (1913).

over 14,000 feet on Pike's Peak, using for this purpose the bag method of DOUGLAS.* On this occasion, four determinations of the total respiratory exchange were made in each experiment at different intervals after the cessation of the muscular exertion, and, by making a considerable number of experiments, it was possible to obtain a fairly complete picture of the course of events in the hour-and-a-half immediately succeeding the muscular exertion. The main disadvantage was that, as the experiments had to be made on different days, the initial values for the resting respiratory exchange and respiratory quotient varied considerably in the different experiments. From a consideration of these experiments, it was, however, evident to us that the bag method could easily be adapted to give a practically continuous record of the respiratory exchange in a single experiment, and that the result would be infinitely more satisfactory than that obtained from a few observations made in each of a number of different experiments.

EXPERIMENTAL METHOD.

The general arrangement of the apparatus used by us is shown in fig. 1. The subject sat on a Krogh bicycle ergometer,† and breathed through a mouthpiece

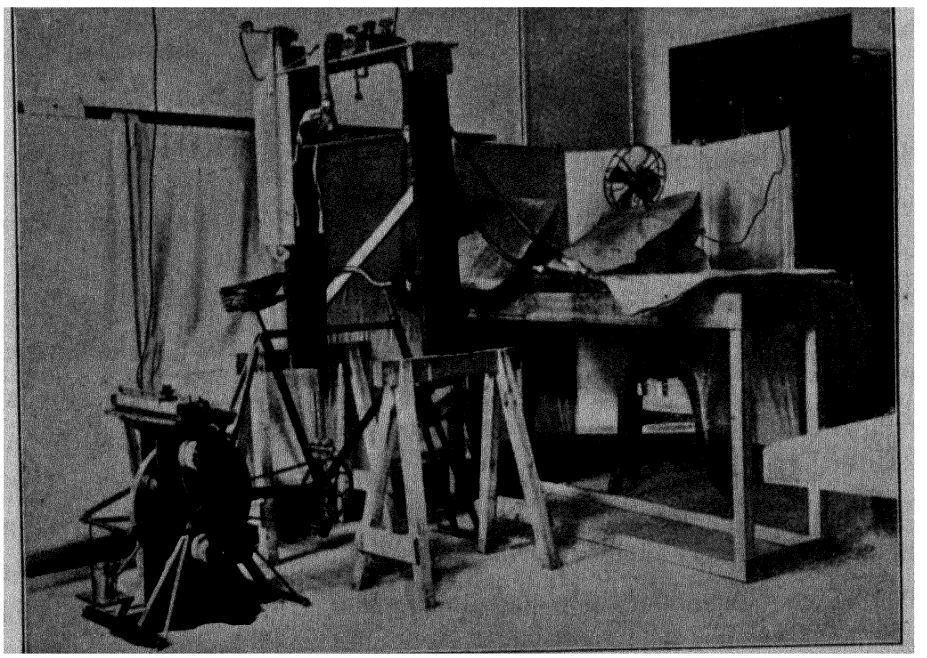


FIG. 1.

connected with inspiratory and expiratory valves. A flexible rubber pipe passed from the expiratory valve to the centre of a piece of brass tubing, into the opposite side, of which were let four short pieces of brass tubing, so that, when the ends of the pipe were included, a six-way distributing system for the expired air was formed.

* 'Journ. Physiol.,' vol. 42; Proc. Physiol. Soc.,' p. xvii (1911).

† 'Skand. Archiv f. Physiol.,' vol. 30, p. 375 (1913).

One end of the main brass tube was closed by a plain rubber stopper, the other by a rubber stopper through which passed a glass tube connected with a tambour writing on a Mackenzie polygraph. The four short pieces of brass tubing were connected to large-bore brass taps, and these in turn by means of lengths of rubber tubing to four gas bags, in which the expired air was collected, the mouth of each gas bag being fitted with a large-bore aluminium tap.* The bore of the rubber and brass tubes was $\frac{3}{4}$ inch. Fig. 2 gives a diagram of the distributing system.

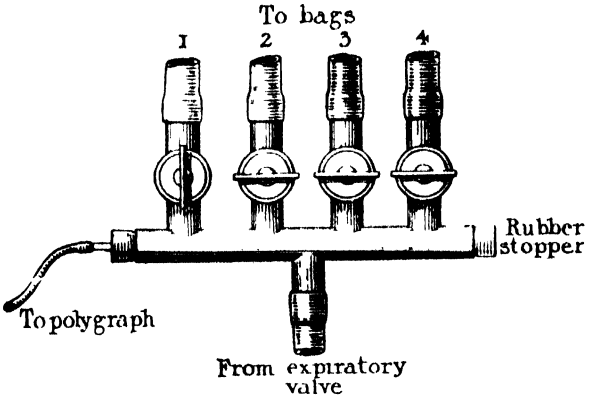


FIG. 2.

Towards the close of the investigations described in this paper, a somewhat different arrangement of the apparatus was devised, and this is shown in fig. 3. In

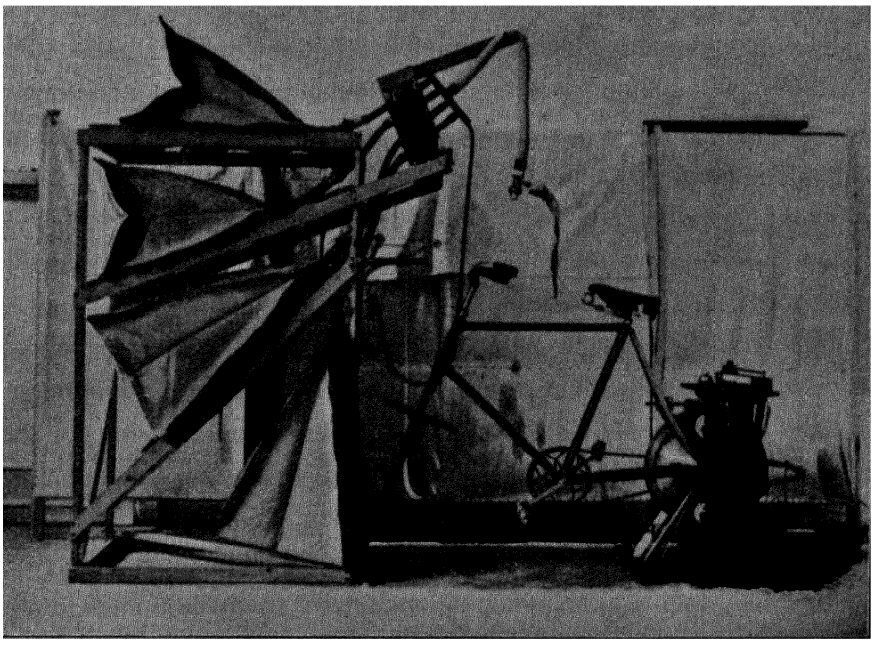


FIG. 3.

* The gas bags, aluminium taps, and valves were made by Messrs. Siebe, Gorman and Co., Westminster Bridge Road, S.E. The valves had been made of specially large bore for us, and offered hardly any appreciable resistance to the passage of even 100 litres of expired air per minute.

this form the bags were mounted one above another in a wooden framework, so that the pipes connecting the distributing system with the bags were reduced considerably in length, and the whole apparatus was rendered far more compact and convenient to work with. All the experiments described in this paper were, however, made with the arrangement shown in fig. 1.

The course of an experiment was as follows: The whole apparatus, including connecting pipes and bags, was first filled with expired air by breathing through the respiration valves. The rubber stopper was then removed from the main brass tube in the distributing system, and the bags emptied in succession by pressing on them and finally rolling them up, the taps on the distributing system being closed directly this was completed. This procedure ensured that any air remaining in the bags and connecting tubes would have approximately the same composition as the expired air entering them during the experiment, and, as the bags were always emptied through the meter in precisely the same way when measuring the volume of expired air collected, any error due to the residual volume of air in the bag was rendered negligible.

The subject then seated himself on the bicycle ergometer with his feet on the foot rests, and maintained himself in as complete a condition of rest as possible, breathing through the valves with a clip on his nose. The expired air passed through the free opening to air in the distributing system. After a preliminary period of 10 or 15 minutes, the rubber stopper was placed in the free opening at the end of an expiration, and one of the taps in the distributing system was turned simultaneously, so that the expired air was diverted into the corresponding bag, the time being noted on a stop-watch. After a sufficient sample had been collected (in about 5–6 minutes), the tap was closed at the end of an expiration and the rubber stopper simultaneously removed, the time being again noted; the subject continued to breathe through the valves to air. The bag, after turning the aluminium tap which closed its mouth, was removed from the tube connecting it with the distributing apparatus and carried to the meter (a wet meter of the Bohr pattern giving 10 litres per revolution), where the volume of expired air collected was measured, after mixing it thoroughly by frequent pressure on the bag, and a small sample was reserved for analysis. After this the empty bag was reattached to the apparatus in readiness for another sample. In some experiments, a second determination of the resting respiratory exchange was made before commencing the muscular work, the total period of rest before starting the work being correspondingly increased. The changes in pressure in the distributing system at each breath were recorded by means of the polygraph, and the tracing therefore gave the number of breaths that were taken while the expired air was being collected.

As soon as the resting respiratory exchange had been determined, the subject commenced to pedal the bicycle against the electric brake. Three different loads were employed in the different experiments, and the muscular work was kept up for

either a shorter or a longer period. The subject kept time in pedalling to a metronome placed just in front of him, set at a speed that he found to be convenient (176 revolutions of the back wheel, or 50 complete revolutions of the pedals per minute). The steadiness of the rate of pedalling was further checked by reading the automatic counter on the ergometer at intervals.

While the muscular exercise was in progress, either two or four determinations of the respiratory exchange, depending on the duration of the exercise, were made at intervals, in the same way as that described above. With the last determination of the respiratory exchange during the exercise, the collection of the samples of expired air became continuous. Directly the bag in which the last sample of expired air was collected during the exercise was full, the observer told the subject to stop working, and simultaneously closed the tap on the distributing system connected with the full bag, and opened the tap connected with one of the other bags. The moment this one was filled, he turned the expired air into the third bag, and so on, always turning the taps at the end of an expiration and noting the time on the stop-watch (we used a stop-watch with a split seconds hand, so as to allow us to note the time accurately without disturbing the continuous record of the time). As the bags were filled, they were carried away and their contents measured, reserving samples for analysis, and they were then replaced on the apparatus ready for use again. As soon as the subject received the word to stop, he replaced his feet on the foot rests and resumed his previous condition of rest, and remained thus until the close of the experiment.

In an experiment, one of us (either DOUGLAS or HOBSON) acted as subject, the second looked after the distributing taps and noted the times, while the third removed the bags as they were filled and measured their contents, noting the temperature and taking samples for subsequent analysis. Even in the experiments which involved the greatest hyperpnœa, we found that it was possible, when using four bags on the apparatus, to complete the measurement of the sample taken just before stopping the work, and to return the bag to the apparatus in plenty of time for the reception of the fourth sample after the cessation of the work. After this, the hyperpnœa had diminished to such an extent that the bags took a considerable time to fill, and measurement of the different samples became easy.

In order to keep the subject cool during the muscular exercise a current of air was allowed to play on him from an electric fan, and in the severe work experiments, two fans were used for this purpose, the fans being turned off soon after the subject ceased to work.

The general accuracy of the Douglas method for determining the respiratory exchange has been proved by CARPENTER* by comparison with different forms of the Benedict apparatus, the Zuntz-Geppert apparatus, and the Tissot apparatus. We

* "A Comparison of Methods for Determining the Respiratory Exchange of Man," 'Publication No. 216, Carnegie Institute of Washington,' 1915.

may note here that we tested the bags we employed to make sure that any diffusion of gas through the walls was negligible. CARPENTER has some distrust of the Siebe Gorman valves, but we took care to test the ones that we used in these experiments, to see whether there was any leakage backwards through the inspiration valve. For this purpose, we did two series of experiments, one at rest, the other at work. In each series determinations of the respiratory exchange were made alternately, firstly with the valves used without any safeguard, and secondly with the addition of a rubber tube 157 cm. long and 2·5 cm. in bore on to the inlet side of the inspiration valve. If there were any material leakage backwards through the valve, the expired air which had leaked out would be held up in the rubber tube, and would be rebreathed at the next breath, and the total respiratory exchange would consequently be found to be greater with the long tube than without it. The results are given in Table I.

TABLE I.

Duration of observation.	Respiratory exchange in c.c. per minute at 0° and 760 mm.		Respiratory quotient.	Breaths per minute.	At 37°, moist, and prevailing barometer.	
	O_2.	CO_2.			Litres breathed per minute.	C.c. per breath.
5′ 47″ rest	297	235	0·791	20·6	10·0	484
*5 7 ,,	297	239	0·805	20·1	10·2	505
5 56 ,,	280	218	0·779	20·2	9·4	465
*6 21 ,,	288	227	0·788	19·8	9·6	485
1 28 work	1605	1538	0·958	25·2	41·7	1654
*1 32 ,,	1600	1506	0·941	25·4	40·8	1606
2 23 ,,	1620	1489	0·919	25·6	40·5	1582
*2 23 ,,	1668	1535	0·920	25·2	41·2	1635

* Samples taken with tube on the inspiration valve.

Rested for 10′, and worked for more than 5′ before beginning to take the samples. Work = 704 kg.m. per minute.

It will be seen that in the rest experiments there are slight differences between the successive results, but there is no definite indication that the results obtained with the addition of the long tube are higher than those without it. In the work experiments the oxygen consumption progressively increases, and the respiratory quotient falls. This, however, is quite characteristic of the respiratory exchange during work in the case of DOUGLAS, who served as the subject in these experiments (see Tables and figures below), and here again there is no distinct indication of any serious loss of the expired air by leakage backwards through the inspiratory valve. We always took care to keep the valves as vertical as possible with the inspiratory valve lowermost—an important point, as the mica discs of the Siebe Gorman valves are closed only by gravity and are not assisted by a spring.

DOUGLAS felt quite comfortable when sitting still on the bicycle, but HOBSON

always found the position rather irksome. This may account for the high respiratory exchange shown by HOBSON when at rest, for this was more than one would have expected even when one allowed for the fact that HOBSON was bigger and of greater muscular development than DOUGLAS. HOBSON was in far better muscular condition than DOUGLAS, who was quite out of training.

All our experiments were commenced about two hours after taking a light breakfast. In some respects this is an undesirable feature, but as BENEDICT and CATHCART'S experiments,* which were made on a subject in the post-absorptive state, *i.e.*, when he had taken no food for the previous 12 hours, show the same type of changes as do our experiments, we can feel tolerably certain that though the food may have influenced the degree of the changes of the respiratory exchange caused by the muscular work in our experiments, it has not seriously influenced their general character.

EXPERIMENTAL RESULTS.

1. *Moderate Work of* 704 *kg.m. per minute.*

(*a*) Short period of muscular work, viz., about ¼ hour.

The results are given in Table II, and Experiment 4 is shown graphically in fig. 4.

These were our earliest experiments, and the data are not altogether complete, Experiments 2 and 3 lacking determinations of the respiratory exchange in the preliminary period preceding the work. During the work the respiratory exchange per minute was about 1700 c.c. of oxygen and 1500 c.c. of carbon dioxide. HOBSON showed higher values than DOUGLAS, but it may be noted that his respiratory exchange at rest was always considerably above that of DOUGLAS. After the stop of the exercise the respiratory exchange falls back extremely rapidly at first, and then more slowly to a value which, so far as can be judged from the available data, corresponds pretty closely with the preliminary resting value obtained just before the exercise commenced.

The respiratory quotient is distinctly raised during the work in Experiment 1 (DOUGLAS), and a similar rise seems probable in Experiment 3; but in Experiment 4 (HOBSON) there is little or no evidence of this rise. Both subjects are alike in showing a marked rise of the respiratory quotient to above unity in the first period following the cessation of the exercise. This rise is, however, only temporary, and the respiratory quotient shows a great diminution in the second period after the stop, and soon attains a normal level. In Experiment 4 (HOBSON), the respiratory quotient seems eventually to drop back to just the same value that it had before the exercise commenced, but in Experiments 1, 2, and 3 (DOUGLAS) there is perhaps a slight indication of a trifling drop in the respiratory quotient below the preliminary resting value and a subsequent recovery.

* BENEDICT and CATHCART, "Muscular Work," 'Publication No. 187, Carnegie Institute of Washington,' 1913.

Table II.

Successive periods of experiment.	Respiratory exchange in c.c. per minute at 0° and 760 mm. O_2.	Respiratory exchange in c.c. per minute at 0° and 760 mm. CO_2.	Respiratory quotient.	Breaths per minute.	At 37°, moist, and prevailing barometer. Litres breathed per minute.	At 37°, moist, and prevailing barometer. C.c. per breath.	CO_2 per cent. in expired air.
Experiment 1.—Douglas. Bar. 764 mm., room temp. 15° C. Work = 704 kg.m. per minute for 14¾ minutes. No artificial cooling.							
10′ 0″ rest	—	—	—	—	—	—	—
4 42 „	286	227	0·793	15·3	7·6	499	3·70
12 0 work	—	—	—	—	—	—	—
2 50 „	1665	1502	0·902	25·1	39·6	1578	4·71
2 8 rest	788	855	1·085	21·6	25·8	1195	4·12
4 0 „	—	—	—	—	—	—	—
5 33 „	—*	—*	0·849	17·5	—*	—*	3·23
7 8 „	328	270	0·823	19·3	10·4	646	3·25
6 11 „	311	248	0·797	15·7	9·7	616	3·20
4 0 „	—	—	—	—	—	—	—
6 0 „	332	254	0·765	—	10·0	—	3·14
6 0 „	299	235	0·786	—	9·6	—	3·07
6 0 „	301	241	0·800	—	9·6	—	3·12
Experiment 2.—Douglas. Bar. 755 mm., room temp. 15·5° C. Work = 704 kg.m. per minute for 17 minutes. No artificial cooling.							
14′ 45″ rest	—	—	—	—	—	—	—
17 0 work	—	—	—	—	—	—	—
2 32 rest	728	772	1·060	20·1	24·3	1210	3·91
2 32 „	403	364	0·903	17·0	12·5	735	3·58
2 25 „	356	322	0·905	17·4	11·4	652	3·49
6 10 „	319	264	0·828	16·4	10·2	619	3·20
7 23 „	316	246	0·779	14·8	9·2	624	3·28
7 0 „	299	226	0·756	15·0	8·9	590	3·15
7 4 „	296	223	0·754	14·9	8·8	590	3·13
6 53 „	303	239	0·789	14·2	9·1	644	3·21
7 1 „	289	228	0·789	16·0	9·2	575	3·05
5 39 „	299	235	0·786	15·2	9·3	611	3·12
Experiment 3.—Douglas. Bar. 753 mm., room temp. 16° C. Work = 704 kg.m. per minute for 14 minutes. Cooled by one fan during work.							
15′ 0″ rest	—	—	—	—	—	—	—
12 0 work	—	—	—	—	—	—	—
2 4 „	1652	1441	0·872	22·7	37·9	1670	4·68
2 0 rest	752	818	1·087	18·0	24·4	1356	4·12
2 2 „	416	356	0·856	11·8	11·6	983	3·78
2 3 „	388	293	0·755	11·7	9·8	834	3·70
5 58 „	319	261	0·819	12·0	9·3	776	3·46
6 0 „	322	253	0·786	11·3	8·9	787	3·50
6 7 „	307	236	0·769	11·6	8·4	723	3·47
6 2 „	309	239	0·773	11·4	8·6	756	3·42
7 0 „	295	230	0·780	12·1	8·6	706	3·32
7 9 „	296	235	0·794	12·6	8·6	681	3·38
7 13 „	284	229	0·807	12·6	8·6	683	3·28

No lactic acid found in either the urine collected immediately before the experiment or in that collected during the experiment.

* Mistake made in measurement of expired air sample.

TABLE II—*continued.*

Successive periods of experiment.	Respiratory exchange in c.c. per minute at 0° and 760 mm.		Respiratory quotient.	Breaths per minute.	At 37°, moist, and prevailing barometer.		CO_2 per cent. in expired air.
	O_2.	CO_2			Litres breathed per minute.	C.c. per breath.	
Experiment 4.—HOBSON. Bar. 768 mm., room temp. 16·5° C. Work = 704 kg.m. per minute for 15 minutes. Cooled by one fan during work.							
5′ 0″ rest	—	—	—	—	—	—	—
5 1 „	385	331	0·860	17·5	10·9	620	3·68
5 3 „	350	308	0·880	16·6	10·1	611	3·66
5 0 work	—	—	—	—	—	—	—
2 33 „	1750	1571	0·898	23·9	36·2	1515	5·22
5 0 „	—	—	—	—	—	—	—
2 33 „	1714	1540	0·899	24·3	36·7	1510	5·05
3 3 rest	595	630	1·059	20·7	18·8	909	4·04
2 32 „	354	322	0·910	20·5	11·4	555	3 42
2 28 „	399	350	0 877	19·1	11 7	613	3·62
7 2 „	370	332	0·898	20·5	11·6	567	3·45
5 30 „	383	344	0·898	19·8	11·5	583	3 60
7 34 „	344	304	0·884	18·6	10 5	565	3·50
6 3 „	335	293	0·875	18·8	10·4	554	3·40
8 18 „	344	301	0·875	18·4	10 6	575	3·44

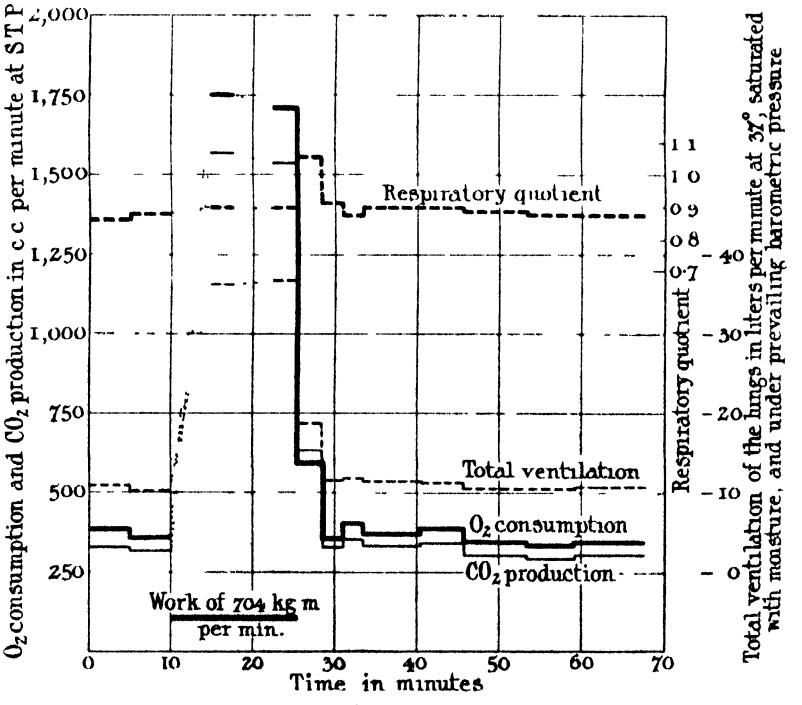


FIG. 4.

(*b*) Long period of muscular work, viz., ¾–1 hour.

The results are given in Table III, and Experiment 6 is shown graphically in fig. 5.

TABLE III.

Successive periods of experiment.	Respiratory exchange in c.c. per minute at 0° and 760 mm.		Respiratory quotient.	Breaths per minute.	At 37°, moist, and prevailing barometer.		CO_2 per cent. in expired air.
	O_2.	CO_2.			Litres breathed per minute.	C.c. per breath.	
Experiment 5.—DOUGLAS. Bar. 764 mm., room temp. 17° C. Work = 704 kg.m. per minute for 45 minutes. Cooled by one fan during work.							
5′ 0″ rest	—	—	—	—	—	—	—
5 1 „	294	239	0·813	11·6	8·2	707	3·53
5 0 „	—	—	—	—	—	—	—
5 1 „	293	231	0·788	10·8	7·8	724	3·58
5 0 work	—	—	—	—	—	—	—
2 15 „	1611	1504	0·934	18·2	37·0	2030	4·93
10 0 „	—	—	—	—	—	—	—
2 33 „	1619	1470	0·908	20·8	37·3	1794	4·77
10 0 „	—	—	—	—	—	—	—
2 32 „	1664	1480	0·890	20·1	37·6	1871	4·76
10 0 „	—	—	—	—	—	—	—
2 34 „	1747	1504	0·861	17·5	37·8	2160	4·82
2 30 rest	717	725	1·011	16·0	21·4	1338	4·10
2 30 „	330	316	0·957	11·6	10·5	905	3·65
2 30 „	302	262	0·867	10·0	8·7	870	3·65
4 0 „	321	276	0·860	10·0	9·3	933	3·59
Experiment 6.—DOUGLAS. Bar. 758 mm., room temp. 15·5° C. Work = 704 kg.m. per minute for 57½ minutes. Cooled by one fan during work.							
10′ 0″ rest	—	—	—	—	—	—	—
5 0 „	263	213	0·808	9·4	7·1	754	3·67
5 0 work	—	—	—	—	—	—	—
2 31 „	1624	1534	0·945	16·3	37·6	2310	4·98
22 30 „	—	—	—	—	—	—	—
2 31 „	1695	1520	0·897	17·1	37·8	2210	4·92
22 30 „	—	—	—	—	—	—	—
2 33 „	1732	1486	0·858	17·2	36·9	2145	4·92
2 31 rest	721	720	0·998	13·9	20·8	1496	4·23
3 3 „	—*	—*	0·846	—	—*	—*	3·71
3 1 „	325	278	0·855	10·9	9·1	830	3·76
5 31 „	286	232	0·812	11·6	8·4	727	3·41
6 58 „	283	220	0·778	10·3	8·0	776	3·38
7 5 „	279	221	0·792	9·7	7·7	793	3·52
7 0 „	277	214	0·772	9·3	7·3	783	3·59
7 8 „	267	209	0·783	9·5	7·5	784	3·43
6 51 „	265	208	0·785	9·1	7·4	815	3·43
7 6 „	268	211	0·787	9·9	7·6	766	3·41

In one hour preceding experiment 47 c.c. urine, sp. gr. 1016. During whole experiment 265 c.c. urine, sp. gr. 1008. Lactic acid test negative in both samples.

* Mistake made in measurement of expired air sample.

TABLE III—*continued.*

Successive periods of experiment.	Respiratory exchange in c.c. per minute at 0° and 760 mm. O_2.	Respiratory exchange in c.c. per minute at 0° and 760 mm. CO_2.	Respiratory quotient.	Breaths per minute.	At 37°, moist, and prevailing barometer. Litres breathed per minute.	At 37°, moist, and prevailing barometer. C.c. per breath.	CO_2 per cent. in expired air.
Experiment 7.—HOBSON. Bar. 773 mm., room temp. 15° C. Work = 704 kg.m. per minute for 53 minutes. Cooled by one fan during work.							
10′ 0″ rest	—	—	—	—	—	—	—
4 43 „	—*	—*	0·866	18·7	—*	—*	3·19
5 0 work	—	—	—	—	—	—	—
2 31 „	1664	1512	0·909	20·3	36·8	1814	4·91
15 0 „	—	—	—	—	—	—	—
2 36 „	1738	1507	0·867	24·6	37·8	1536	4·77
13 0 „	—	—	—	—	—	—	—
2 34 „	1687	1487	0·882	24·9	37·9	1521	4·69
10 0 „	—	—	—	—	—	—	—
2 33 „	1676	1481	0·885	24·7	37·8	1531	4·69
2 20 rest	836	863	1·031	24·4	28·4	1164	3·64
2 19 „	384	339	0·883	19·9	12·8	642	3·18
2 6 „	396	311	0·785	19·5	11·7	601	3·18

* Mistake made in measurement of expired air sample.

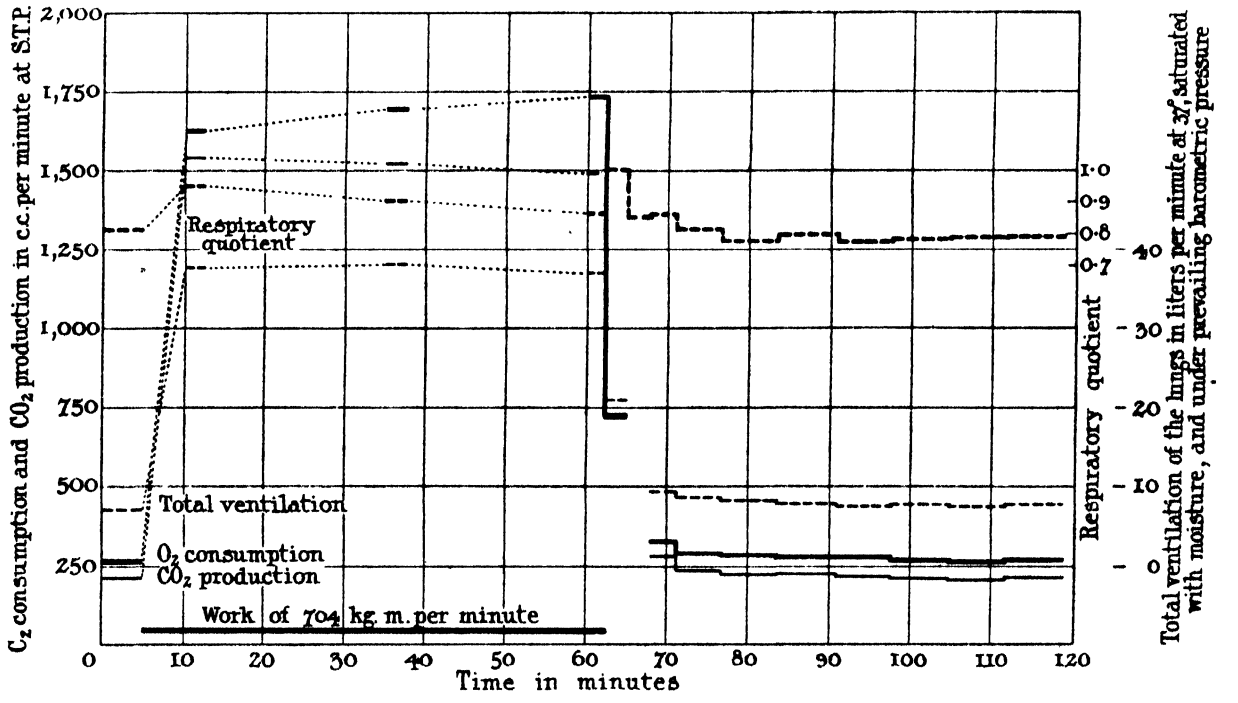


FIG. 5.

The average respiratory exchange during the exercise is practically the same as in the experiments with the shorter period of work, and there is the same rapid fall in the respiratory exchange on cessation of the exercise, and the same temporary sharp rise of the respiratory quotient immediately after the stop.

In Experiment 7 (HOBSON) the respiratory exchange remains tolerably steady throughout the whole period of work, the respiratory quotient being perhaps raised a trifle during this period, but the case is different in Experiments 5 and 6 (DOUGLAS). In these two experiments, while CO_2 output remains pretty constant during the whole period of exercise, or even falls slightly, the oxygen intake rises steadily throughout the work, until in the last determination made in the work period the oxygen consumption is over 100 c.c. per minute higher than in the first. The respiratory quotient which was markedly raised in the first determination during work in these two experiments on DOUGLAS, falls therefore steadily during the work period, though the final value of this quotient just before stopping the work is still very definitely above the resting value.

The respiratory exchange after the stop of the exercise was only followed for a considerable time in Experiment 6, and in this case there is apparently a slight diminution of the respiratory quotient below the initial resting value after the transitory high quotient immediately following the stop of the work has disappeared, and it is dubious whether there is any tendency to recovery of the original value during the period that the observations were kept up.

The volume of air breathed per minute in these experiments varies in a similar manner to the respiratory exchange, though the oxygen consumption shows a more rapid drop after the stop of the exercise than does either the CO_2 output or the hyperpnœa. During the work the total ventilation of the lungs remains pretty steady, and the increase in the ventilation of the lungs is brought about more by increasing the depth of the respirations than by increasing their rate, for the volume of each breath is increased to about thrice the initial resting value in the case of DOUGLAS (Experiments 1, 5, and 6), and the rate is less than doubled; while in the case of HOBSON (Experiment 4) these values are respectively $2\frac{1}{2}$ times and $1\frac{1}{2}$ times the resting values. An increase of depth of the breathing is, of course, a more economical method of increasing the alveolar ventilation than is an increase of rate, owing to the greater proportional influence of the dead space in shallow breathing, and one finds therefore in these experiments that though the metabolism, as judged by the oxygen consumption has about six times the resting value in the case of DOUGLAS, and $4\frac{1}{2}$ times the resting value in the case of HOBSON, the total ventilation of the lungs is only increased to five times and $3\frac{1}{2}$ times the resting values respectively.

2. *Hard Work of* 1056 *kg.m. per Minute.*

(*a*) Short period of muscular work, viz., 15 minutes.

The results of experiment on DOUGLAS are given in Table IV.

In this case the CO_2 production during the work remains steady, but the oxygen consumption is considerably higher in the second determination than in the first. Though the respiratory exchange falls after the stop of the exercise with a rapidity comparable with that in the previous experiments, there are some significant altera-

tions in the respiratory quotient. In the first place, there is the abnormally high respiratory quotient of unity in the first observation made in the work period, and in the second, the respiratory quotient, after the transitory sharp rise immediately after the stop of the exercise has passed away, falls to a value which is definitely below that which it had before the work was begun, and this value remains practically steady during the last four or five periods of the experiment. The respiratory exchange reaches a steady value 18 minutes after the stop of the exercise, and it will be seen that though the CO_2 output during the remaining 25 minutes of the experiment (a period that corresponds with the persistent low respiratory quotient), is below the value obtained during the preliminary rest period, the oxygen consumption is on the average somewhat above the initial resting value.

TABLE IV.

Successive periods of experiment.	Respiratory exchange in c.c. per minute at 0° and 760 mm.		Respiratory quotient.	Breaths per minute.	At 37°, moist, and prevailing barometer.		CO_2 per cent. in expired air.
	O_2.	CO_2.			Litres breathed per minute.	C.c. per breath.	
Experiment 8.—DOUGLAS. Bar. 764 mm., room temp. 12° C. Work = 1056 kg.m. per minute for 15 minutes. Cooled by two fans during work.							
10′ 0″ rest		—	—	—	—	—	—
5 45 „	281	233	0·829	10·4	8·1	776	3·50
5 0 work	—	—	—	—	—	—	—
1 45 „	2240	2245	1·002	24·6	59·3	2410	4·58
7 0 „	—	—	—	—	—	—	—
1 34 „	2350	2250	0·957	23·3	59·7	2460	4·57
5 5 rest	631	738	1·170	16·7	25 9	1551	3·45
2 58 „	364	324	0·891	14·2	12·4	874	3·17
5 9 „	350	301	0 860	13·4	11 5	857	3·18
4 46 „	331	267	0·806	12·6	10·1	803	3·20
5 2 „	291	229	0·787	11·7	9·1	775	3·07
4 47 „	290	222	0·765	10·9	8·6	789	3·13
5 1 „	275	211	0·767	10·2	8·1	792	3·17
4 52 „	298	225	0·755	12·1	8·9	731	3 09
5 24 „	283	225	0·788	11·1	9·0	807	3 02

In one hour preceding experiment 106 c.c. urine, sp. gr. 1011 ; lactic acid negative.
During whole experiment 245 c.c. urine, sp. gr. 1004, containing 0·10 grm. lactic acid.

It will be noted that in this case the first resting period after the stop of the exercise is much longer than in the other experiments. As a matter of fact there were two periods, but on switching the expired air from the first bag into the second the watch was by accident not stopped, though the approximate position of the hand was noted, and on this occasion it was impossible to distinguish the transition from the first to the second bag on the polygraph tracing of the respirations. In order to reckon the respiratory exchange accurately, the two periods had therefore to be taken together. The first sample took approximately 1½ minutes to collect, and

accepting this figure the respiratory exchange averaged in the first 1½ minutes after the stop of the exercise 1369 c.c. O_2 and 1714 c.c. CO_2 per minute, with R.Q. = 1·254, and in the succeeding 3′ 35″ 324 c.c. O_2 and 329 c.c. CO_2 per minute, with R.Q. = 1·016.

(*b*) Long period of muscular work, viz., 29–38 minutes.

The results are given in Table V, and Experiment 9 is shown graphically in fig. 6.

TABLE V.

Successive periods of experiment.	Respiratory exchange in c.c. per minute at 0° and 760 mm.		Respiratory quotient.	Breaths per minute.	At 37°, moist, and prevailing barometer.		CO_2 per cent. in expired air.
	O_2.	CO_2.			Litres breathed per minute.	C.c. per breath.	
Experiment 9.—DOUGLAS. Bar. 763 mm., room temp. 12° C. Work = 1056 kg.m. per minute for 37 minutes. Cooled by two fans during work.							
10′ 0″ rest	—	—	—	—	—	—	—
5 0 „	308	266	0·863	11·8	9·0	761	3·60
3 0 work	—	—	—	—	—	—	—
1 42 „	2234	2446	1·095	25·3	65·6	2595	4·52
9 0 „	—	—	—	—	—	—	—
1 56 „	2380	2260	0·950	23·8	59·5	2500	4·61
9 0 „	—	—	—	—	—	—	—
1 45 „	2395	2270	0·948	26·0	63·1	2430	4·36
9 0 „	—	—	—	—	—	—	—
1 54 „	2420	2220	0·917	24·7	59·6	2415	4·52
2 1 rest	1070	1258	1·175	30·3	44·5	1470	3·44
3 8 „	396	367	0·927	16·0	14·2	889	3·14
5 47 „	358	301	0·841	13·1	11·4	872	3·20
10 15 „	321	249	0·776	12·1	9·8	808	3·10
11 57 „	324	248	0·765	12·0	9·2	768	3·27
10 52 „	335	253	0·755	12·7	9·4	743	3·26
11 5 „	324	241	0·744	12·8	9·1	707	3·24
10 31 „	334	254	0·760	11·4	9·2	808	3·35
3 0 „	—	—	—	—	—	—	—
10 57 „	330	243	0·736	10·0	8·5	850	3·47
In 1¼ hours preceding experiment 302 c.c. urine, sp. gr. 1004 ; lactic acid negative. During whole experiment 193 c.c. urine, sp. gr. 1011, containing 0·05 grm. lactic acid.							
Experiment 10.—HOBSON. Bar. 755 mm., room temp. 16° C. Work = 1056 kg.m. per minute for 28¾ minutes. Cooled by two fans during work.							
5′ 0″ rest	—	—	—	—	—	—	—
5 21 „	394	349	0·885	19·3	12·7	658	3·38
7 0 „	—	—	—	—	—	—	—
5 11 „	423	373	0·881	19·1	12·9	677	3·55
4 0 work	—	—	—	—	—	—	—
1 49 „	2490	2490	1·000	25·3	59·0	2330	5·17
7 0 „	—	—	—	—	—	—	—
1 59 „	2580	2270	0·880	25·7	55·7	2170	5·00
12 0 „	—	—	—	—	—	—	—
1 52 „	2680	2325	0·868	25·7	54·9	2140	5·20
3 12 rest	832	917	1·102	23·8	28·9	1214	3·90
4 15 „	487	395	0·811	21·7	14·6	624	3·32
4 37 „	464	405	0·872	20·6	14·6	707	3·42
Bicycle saddle too low and subject very uncomfortable throughout the work.							

TABLE V—*continued.*

Successive periods of experiment.	Respiratory exchange in c.c. per minute at 0° and 760 mm.		Respiratory quotient.	Breaths per minute.	At 37°, moist, and prevailing barometer.		CO_2 per cent. in expired air.
	O_2.	CO_2.			Litres breathed per minute.	C.c. per breath.	
Experiment 11.—HOBSON. Bar. 765 mm., room temp. 15° C. Work = 1056 kg.m. per minute for 38 minutes. Cooled by two fans during work.							
10′ 0″ rest	—	—	—	—	—	—	—
6 33 „	375	323	0·860	16·9	11·3	671	3·45
3 6 work	—	—	—	—	—	—	—
1 55 „	2310	2230	0·966	25·0	53·9	2125	5·01
9 10 „	—	—	—	—	—	—	—
1 50 „	2435	2250	0·925	25·1	56·5	2250	4·82
9 0 „	—	—	—	—	—	—	—
1 46 „	2400	2240	0·934	24·9	56·7	2280	4·78
9 26 „	—	—	—	—	—	—	—
1 47 „	2360	2140	0·907	24·7	54·2	2190	4·78
2 39 rest	819	852	1·041	23·4	26·7	1141	3·87
3 7 „	426	389	0·913	22·8	14·3	629	3·29
3 22 „	376	337	0·896	22·0	12·8	582	3·20
7 29 „	395	331	0·838	21·4	12·4	579	3·24
7 53 „	371	305	0·822	20·7	11·7	567	3·15
8 19 „	355	293	0·826	19·7	11·2	569	3·17
8 46 „	358	295	0·824	20·1	11·1	550	3·24
7 40 „	334	269	0·805	18·9	10·3	542	3·18
8 10 „	350	283	0·809	19·5	10·6	541	3·26
During the whole of the experiment and in the hour preceding it 95 c.c. urine, sp. gr. 1023, which showed only a trace of lactic acid.							

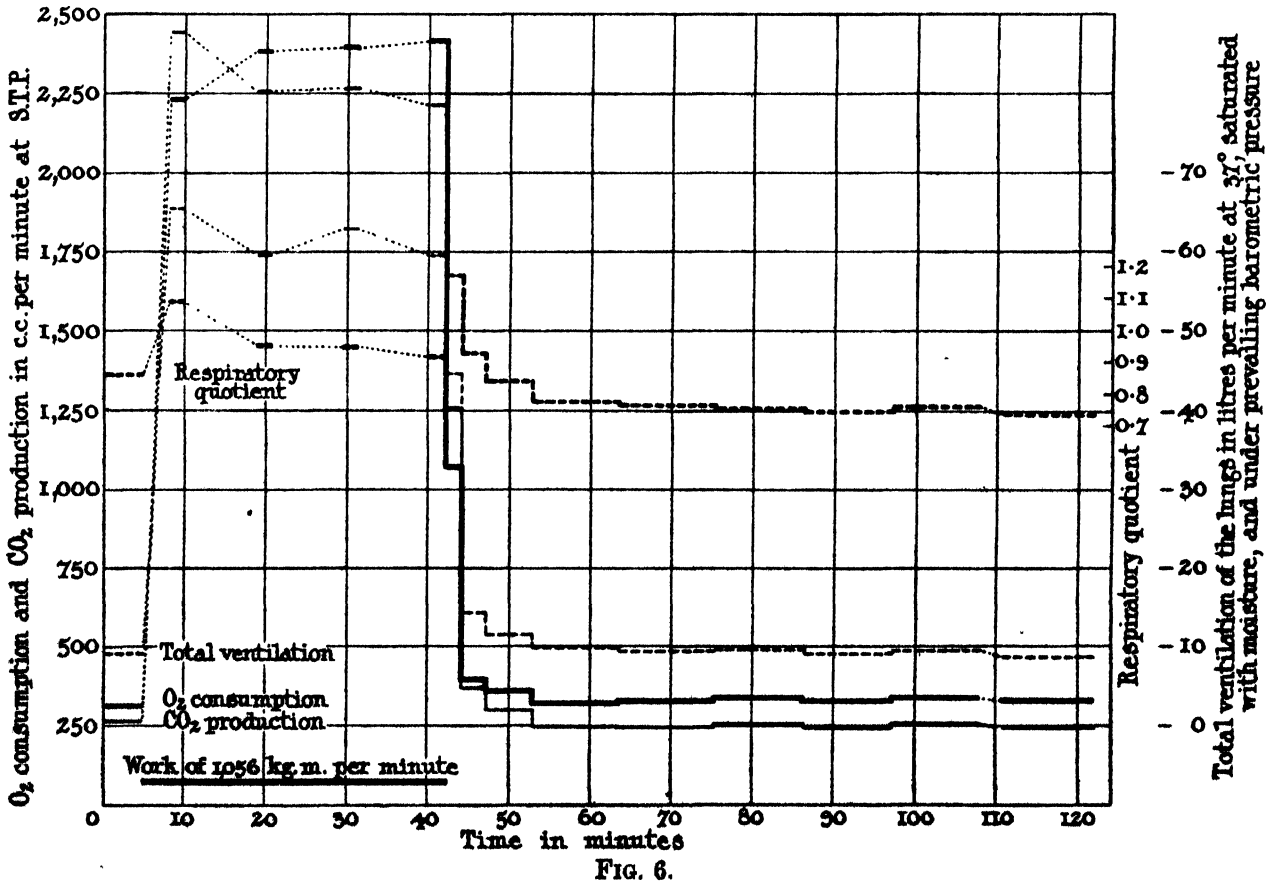


FIG. 6.

To deal first with Experiment 9 (DOUGLAS). The oxygen consumption in the first two determinations during the work period agrees closely with that found in Experiment 8. It shows a marked rise during the course of the work, and this rise is greater than was the case in Experiments 5 and 6, the oxygen consumption being 186 c.c. per minute higher in the last determination made during the work period than it was during the first. The CO_2 output is 112 c.c. per minute above the oxygen intake in the first work period, and the respiratory quotient well above unity at this time (it should be noted that the sample was taken at an earlier stage of the work period than in Experiment 8). The CO_2 output has dropped below the oxygen consumption by the time of the second observation during the work and remains fairly steady, or even diminishes slightly, during the remainder of the exercise, the respiratory quotient, which had fallen to 0·95 in the second observation, diminishing further as the oxygen consumption increases, though it is still well above the preliminary resting value in the last period of work.

After the stop of the exercise there is the usual immediate rise of the respiratory quotient followed by a rapid drop. The respiratory exchange falls rapidly, and at the end of 11 minutes has reached a value which remains practically constant for the subsequent 68½ minutes of the experiment. During this last 68½ minutes the respiratory quotient remains pretty steady at a figure considerably below the initial resting value shown at the commencement of the experiment, the diminution of the respiratory quotient being proportionally greater than in Experiment 8. The low respiratory quotient is due to the fact that the CO_2 output is on the average 18 c.c. per minute below the initial resting value as well as to the fact that the oxygen consumption is 20 c.c. per minute above the initial resting value. It will, moreover, be seen that the average oxygen consumption of 328 c.c. per minute during this period is considerably above what was found in the previous experiments on DOUGLAS either during the preliminary rest period or in the later stages of rest subsequent to muscular work when the respiratory exchange has reached a steady value.

To allow of comparison with Experiment 8, the first and second periods after the stop of the work may be added together. If this is done the oxygen consumption averaged 660 c.c. per minute, and the CO_2 output 716 c.c. per minute, with respiratory quotient 1·085, during the first 5′ 9″ after stopping the work.

In this experiment DOUGLAS was quite comfortable throughout the work, and though he felt a little tired at the end he could have continued the work for a good deal longer without serious discomfort.

Experiment 10 (HOBSON) gives a picture very similar to Experiment 9. There is the same steady rise in the oxygen consumption during the work period, the oxygen consumption being 200 c.c. per minute higher in the third determination than in the first, while the CO_2 output is 220 c.c. per minute lower in the second determination during the work, and 155 c.c. per minute in the third, than it was in the first. The respiratory quotient is 1·0 in the first determination during the work,

but in the second and third determinations it falls to a value identical with the initial resting figure. In this experiment HOBSON forgot to raise the bicycle saddle to the proper height, and in consequence found the work extremely uncomfortable. We were therefore compelled to terminate the work earlier than we had intended.

The experiment was therefore repeated on HOBSON some days later (Experiment 11), with the bicycle saddle at the correct height. This time he was pretty comfortable throughout the work, though he sweated a good deal notwithstanding the two fans. The picture presented in this experiment is somewhat different from that in the preceding one. The average respiratory exchange during the work is a good deal lower, as indeed one might expect since he was not subject to the disadvantage of a cramped position. Though the oxygen consumption in the three later observations during the work is higher than in the first, the rise is neither so marked as in Experiment 10 nor is it progressive. The CO_2 output remains constant for the greater part of the work period, but diminishes somewhat in the last determination. The respiratory quotient is only 0·966 in the first observation during the work, and shows a diminution during the course of the work, though in the last observation during the work it is still a good deal above the initial resting value. The whole course of events during the muscular work is, in fact, remarkably like that found in the case of DOUGLAS in the long experiments with lighter work (Experiments 5 and 6, Table III). Nine minutes after the work ceased, however, the respiratory quotient falls below the initial resting value, and a further fall occurs in subsequent periods, and even when the experiment terminated 48 minutes later there was no sign of recovery.

In Experiments 9 and 10 it will be seen that the hyperpnœa during the work is at a maximum in the first determination, *i.e.*, at a time corresponding to the abnormally high respiratory quotient, and CO_2 output, and that there is a decided drop in the amount of air breathed per minute in the subsequent observations during the exercise. This definite variation in the hyperpnœa is, however, absent in Experiment 11 in which the respiratory quotient during the work is always well below unity. If we disregard for the moment the observations which show an abnormally high respiratory quotient of 1·0 or over during the work in these experiments, since these are evidently influenced by some disturbing factor, and confine ourselves to the period during which the hyperpnœa remains fairly steady, it will be seen that in DOUGLAS'S case the oxygen consumption is increased about eight-fold during the work, while the amount of air breathed per minute is only about seven times the resting value, the rate of breathing being rather more than doubled, and the depth of the breathing rather more than trebled. HOBSON'S oxygen consumption rises during the work to rather more than six times the preliminary resting value, but the total ventilation of the lungs is barely five times the resting value, the rate of breathing being about 1½ times as great, and the depth of breathing more than three times as great, as during rest. The hyperpnœa is therefore being brought about, just as was the case

with the lighter work, more by increasing the depth than by increasing the rate of the respiration. That the hyperpnœa in the later stages of the work in these experiments is not excessive in comparison with that observed in the case of lighter work, is shown by the fact that the average volume of expired air per 1 c.c. of CO_2 given off is in DOUGLAS'S case, 25·2 c.c. in the experiments with work of 704 kg.m. per minute, and 26·9 c.c. in the experiments at 1056 kg.m. per minute, HOBSON giving values of 24·6 c.c. and 24·7 c.c. respectively (observations which show an abnormally high respiratory quotient being excluded as before).

3. *Severe Muscular Work of* 1232 *kg.m. per Minute.*

The results of this experiment (No. 12) are given in Table VI, and are shown graphically in fig. 7.

TABLE VI.

Successive periods of experiment.	Respiratory exchange in c.c. per minute at 0° and 760 mm.		Respiratory quotient.	Breaths per minute.	At 37°, moist, and prevailing barometer.		CO_2 per cent. in expired air.
	O_2.	CO_2.			Litres breathed per minute.	C.c. per breath.	
Experiment 12.—DOUGLAS. Bar. 762 mm., room temp. 16° C. Work = 1232 kg.m. per minute for 7¾ minutes. Cooled by two fans during work.							
21′ 27″ rest	—	—	—	—	—	—	—
4 0 work	—	—	—	—	—	—	—
4 0 rest	—	—	—	—	—	—	—
3 0 work	—	—	—	—	—	—	—
0 45 „	2795	2880	1·030	48·0	114·8	2390	3·06
0 51 rest	2110	2390	1·132	56·5	105·1	1862	2·77
1 37 „	806	1011	1·255	33·4	44·4	1330	2·78
3 13 „	469	485	1·033	20·8	20·6	991	2·87
7 2 „	379	359	0·947	19·5	16·1	826	2·72
9 4 „	334	268	0·802	18·2	12·7	699	2·58
6 40 „	325	240	0·739	19·5	11·6	596	2·53
9 24 „	292	199	0·684	17·7	9·7	550	2·50
11 31 „	276	178	0·645	18·7	9·1	489	2·38
10 2 „	318	207	0·651	18·7	10·0	534	2·53
4 42 „	—	—	—	—	—	—	—
10 31 „	330	214	0·648	18·0	9·8	545	2·67
10 54 „	323	221	0·684	19·0	9·9	521	2·62
In one hour preceding experiment 34 c c. urine, sp. gr. 1026 ; lactic acid negative. During whole experiment 92 c.c. urine, sp. gr. 1022, containing 0·57 grm. lactic acid.							

This amount of work proved too severe for the subject (DOUGLAS) to keep it up for more than a few minutes. The initial rest period lasted for 21½ minutes, but, owing to an error, we did not succeed in determining the respiratory exchange during this time. We had intended that the work should be kept up for five minutes before collecting the first sample of expired air, but, at the end of four minutes, the hyperpnœa and distress were so great that the subject could no longer tolerate

breathing into the apparatus, and he was forced to stop working. After an interval of four minutes' rest, at the end of which time the hyperpnœa had greatly diminished, though it had by no means ceased, the work was begun again. Three minutes later the respiratory exchange was determined over a period of 45 seconds, and then the subject stopped work. The after-effect of the work was followed for 85½ minutes.

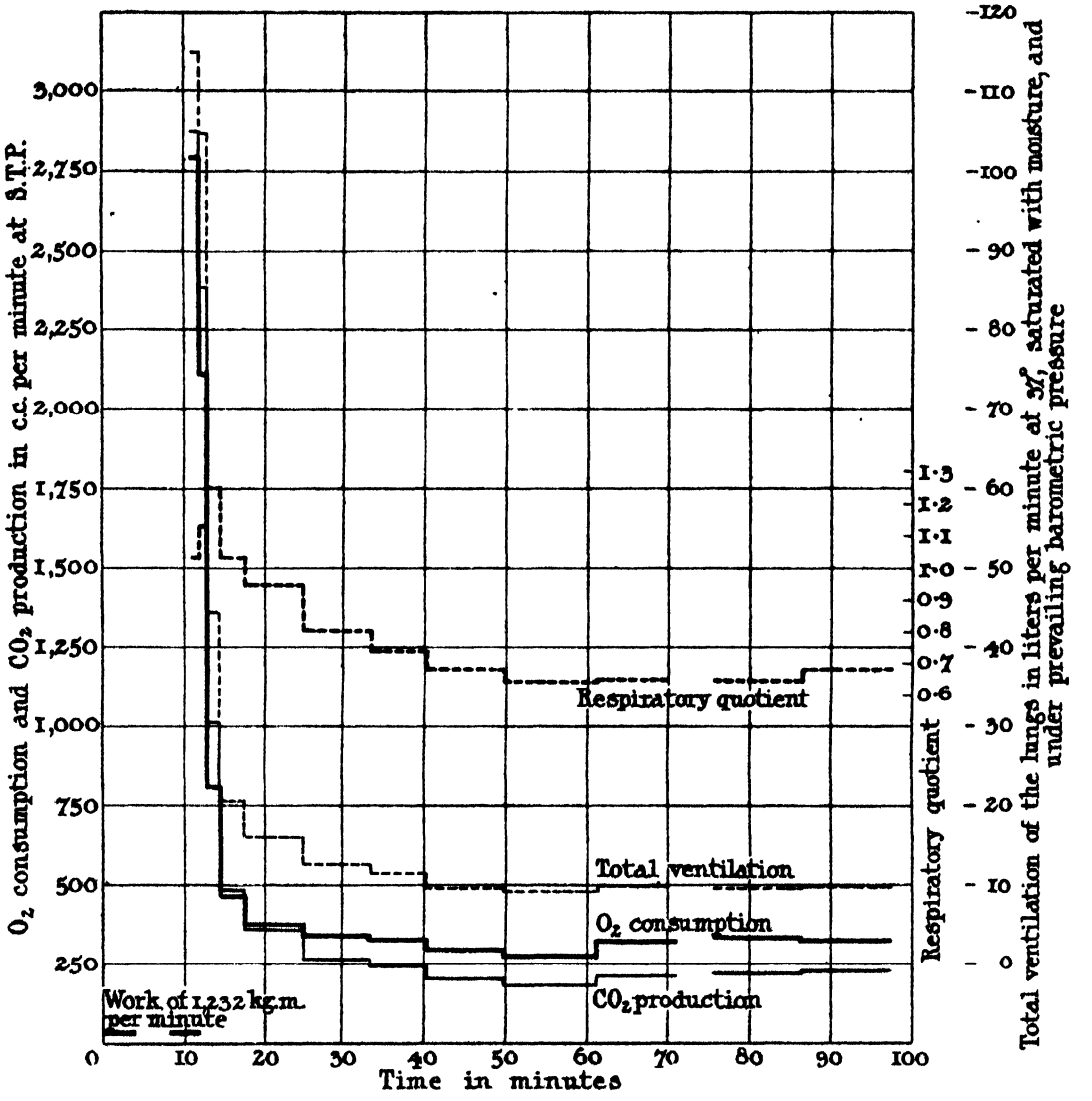


FIG. 7.

It will be seen that, in spite of the fact that the work had already lasted for a total of seven minutes, and that there had been, in addition, an interval of four minutes between the two periods of work, the CO_2 output is still above the oxygen consumption, and the respiratory quotient above unity, when the determination of the respiratory exchange was made just before stopping the work.

In the first minute after the stop of the work, there is only a comparatively small drop in the respiratory exchange, though there is a big diminution in the succeeding minute and a-half, and a considerable further fall in the next three minutes. The

figures show clearly how greatly the fall in the CO_2 output lags behind the fall in the oxygen consumption during these three periods. The respiratory exchange and respiratory quotient fall to a minimum in the eighth period after the stop (*i.e.*, between the 38th and 49th minutes), and subsequently rise again to some extent. It is possible that the lowness of the figures in the eighth period is due to some chance error, though we were unable to identify any fallacy in our measurements, and one may note that as low, or even lower, figures for oxygen consumption are recorded in Experiments 6 and 8. Taking the last 57 minutes of the experiment (seventh period after the stop of the work onwards), as a whole, the striking features are the great depression of the respiratory quotient, which lies well below 0·7, and the height of the oxygen consumption, in comparison with the lowness of the CO_2 output. Lower figures for CO_2 output in individual periods after the work are recorded during this time than in any of the other experiments on DOUGLAS, though the average CO_2 output for the whole period is approached in the later stages of Experiment 6.

The extreme hyperpnœa during the muscular work is obvious. Though the metabolism, as judged by the oxygen consumption, is only about ten times the average preliminary resting value, as opposed to the eight-fold increase shown in Experiments 8 and 9, the volume of air breathed per minute is practically double what it was in these experiments. What is more, this additional increase is brought about entirely by increasing the rate of the respiration, for the depth of the breathing is actually somewhat less than in Experiments 8 and 9. The excessive character of the breathing is well brought out by the extremely low percentage of CO_2 in the expired air (3 per cent., as opposed to $4\frac{1}{2}$ per cent. or more in the previous experiments), as well as by the fact that the volume of expired air per 1 c.c. of CO_2 given off is 39·8 c.c., instead of the 26·9 c.c. observed in the experiments with work of 1056 kg.m. per minute; had the latter ratio held good in Experiment 12, the volume of air breathed per minute would have been only 77·5 litres instead of 114·8. The volume of air breathed per minute remains about as great during the first minute after the stop of the work as it was during the work. It then drops slowly, and attains a fairly steady value $28\frac{1}{2}$ minutes after the cessation of the work. It will be noticed that the rate of the breathing during the later stages of the after-period remains much higher than in the other experiments on DOUGLAS, and that there is a corresponding reduction in the depth; a rate of 18 breaths a minute during rest has, however, often been observed in DOUGLAS under other circumstances.

DISCUSSION OF THE RESULTS.

(1) *The "Efficiency" during the Work.*

Though these experiments were not undertaken with the idea of determining accurately the mechanical efficiency of the body during the performance of muscular

work, *i.e.*, the relationship between the heat equivalent of the work done and the amount of heat actually liberated in the body in the performance of that work, it is of interest to calculate this value in order to compare the results with those obtained in the recent careful experiments of BENEDICT and CATHCART (*loc. cit.*), in which the muscular work was done by a trained subject in the post-absorptive state on a bicycle ergometer, and the energy output was calculated from the respiratory exchange. This may be done by using ZUNTZ and SCHUMBURG's figures* for the calorie value of oxygen at different respiratory quotients, though this method does not, of course, take into account the metabolism of protein.

In order to calculate the efficiency, one must subtract from the total heat output during the work a quantity corresponding to the "basal metabolism," on which the actual heat output corresponding to the work may be assumed to be superimposed. BENEDICT and CATHCART have discussed in detail the different values which may be selected to represent the basal metabolism. We give in Table VII the efficiency values for our experiments, using as "basal metabolism" the energy output in the preliminary rest period when sitting still on the bicycle.

TABLE VII.

	Respiratory exchange in c.c. per minute at 0° and 760 mm.		Respiratory quotient.	Calories produced per minute.	Extra calories per minute during work.	Calorie equivalent of work.	Efficiency.
	O_2.	CO_2.					
DOUGLAS.							per cent.
Preliminary rest . .	288	235	0·816	1·39			
704 kg.m. per minute	1668	1493	0·896	8·21	6·82	1·65	24·2
1056 „ „	2386	2250	0·943	11·91	10·52	2·47	23·5
HOBSON.							
Preliminary rest . .	385	337	0·873	1·88			
704 kg.m. per minute	1705	1516	0·890	8·38	6·50	1·65	25·4
1056 „ „	2398	2210	0·922	11·87	9·99	2·47	24·7

In this Table, the respiratory exchange at rest of each subject is the average of all the values obtained on that subject before starting the work. The figures given with work of 704 kg.m. per minute are the average of all the observations made on the subject in question while this work was in progress. In the case of the work of 1056 kg.m. per minute, the figures only give the average value for the second observation during work in Experiment 8, and the last three observations during work in Experiment 9 on DOUGLAS, and the average value for the last three observations in Experiment 11 on HOBSON. Owing to the liability of the respiratory

* ZUNTZ und SCHUMBURG, 'Physiologie des Marsches,' Berlin, 1901, p. 361.

quotient to be abnormally high in the early stages with this degree of work, we must clearly discard at least the first observations during work in these experiments. We have not included Experiment 10 on HOBSON in the above Table, for it is not strictly comparable with the other experiments, because of the discomfort during the work, owing to the low position of the saddle, but this experiment shows an appreciably lower value for the efficiency than does Experiment 11, the heat equivalent of the oxygen consumption in the last two determinations during work being 12·87 calories.

In the case of DOUGLAS, previous determinations of the respiratory exchange at rest in bed immediately after waking in the morning,* have shown an oxygen consumption per minute of 237 c.c. and a CO_2 production of 197 c.c., with a respiratory quotient of 0·829, corresponding to a heat output of 1·15 calories per minute. Using this value for the "basal metabolism," the efficiency at 704 kg.m. per minute becomes 23·4 per cent., and at 1056 kg.m. per minute 23·0 per cent.

We made two determinations on DOUGLAS on the bicycle ergometer, when he was pedalling at the same speed as during the work, but with no load on the brake, and found an oxygen consumption per minute of 494 c.c. and a CO_2 production of 409 c.c., with a respiratory quotient of 0·828, corresponding to a heat production of 2·39 calories per minute. With this "basal value," which has the advantage of including the heat production involved in merely rotating the pedals, so that any extra heat production during the work corresponds more nearly simply with the output of energy entailed by putting the load on the brake, the efficiency at 704 kg.m. per minute becomes 28·4 per cent., and at 1056 kg.m. 25·9 per cent.

In whichever of these three ways the efficiency is calculated, the results are of much the same order of magnitude as those obtained by BENEDICT and CATHCART when using similar values for the "basal metabolism." If, however, the efficiency is calculated from the difference between the data obtained for each subject at the two different degrees of work, *i.e.*, for the external work of 352 kg.m. per minute, when this is superimposed on external work of 704 kg.m. per minute, the efficiency has the rather low value of 22·2 per cent. for DOUGLAS and 23·5 per cent. for HOBSON.

In those experiments in which the oxygen consumption rises progressively during the work, there is a corresponding increase in the heat production, *i.e.*, a falling off of efficiency, though the fall of efficiency is not quite proportional to the rise of oxygen consumption owing to the alteration of the respiratory quotient. For instance, in Experiment 6 on DOUGLAS, the heat production per minute during the work is 8·10 calories at the first observation, 8·35 at the second, and 8·45 at the third, and there is the same type of alteration in Experiment 5. This effect suggests the influence of fatigue, the more so, as HOBSON, who was in better muscular condition than DOUGLAS, only showed this phenomenon in Experiment 10, in which he was considerably hampered by the low position of the bicycle saddle, and had in

* DOUGLAS, HALDANE, HENDERSON, and SCHNEIDER, *loc. cit.*

consequence an exceptionally high rate of metabolism. A similar slight rise of heat production during the course of muscular work is shown in a few of BENEDICT and CATHCART'S experiments, but it is noticeably absent in the majority, especially in an experiment in which a trained cyclist rode the bicycle ergometer continuously for a period of 4 hours and 22 minutes, during the whole of which time his oxygen consumption per minute was close on 2000 c.c. One may note, too, that the heat production was practically constant in Experiment 9 on DOUGLAS in the last three observations during the work, a period of 23½ minutes, though the oxygen consumption rose considerably between the first and second observations. ZUNTZ and SCHUMBURG* have found a decreased efficiency during walking, as the result of a long march, which they ascribe to the effects of fatigue, but BENEDICT and MURSCHHAUSER† have not been able to find definite evidence of this, though the distance walked reached as much as 14 miles; the pace, however, in their longest experiments was only about 3 miles per hour.

(2) *The Respiratory Exchange during the Work.*

We made no attempt to determine the respiratory exchange during the very early stages of the work, though it would naturally have been of great interest to follow the transition from rest to work as well as from work to rest. We felt, however, that as it takes a brief time for the subject to pick up the rate of the metronome and to steady down to the work, the results would be somewhat difficult to compare with those obtained at a later stage, and we preferred therefore to allow from three to five minutes to elapse after starting pedalling before we took our first samples, so as to allow the subject to get into reasonable equilibrium with the work.

(i) *Work of* 704 *kg.m. per minute.*

While DOUGLAS invariably shows a definite rise of the respiratory quotient throughout the period of work, HOBSON'S respiratory quotient remains practically steady at the same level as in the preliminary resting period. DOUGLAS'S respiratory quotient is highest in the earliest period of work, and diminishes slowly and steadily as the work is continued, though it is considerably above the preliminary resting value even when the work has been kept up continuously for ¾–1 hour.

Such a rise of the respiratory quotient might be determined by one or more of several causes. Thus it might be an indication of a real alteration in the character of the metabolism, and imply that during work a greater proportion of the necessary energy is derived from carbohydrate than during rest. On the other hand, the explanation might be found in some cause which leads to an expulsion of CO_2 from the store held in combination in the body (the so-called "preformed" CO_2) without

* *Loc. cit.*, p. 259.

† BENEDICT and MURSCHHAUSER, "Energy Transformations during Horizontal Walking," 'Publication No. 231, Carnegie Institute of Washington,' 1915.

any corresponding variation in oxygen consumption, or necessary alteration in the character of the metabolic processes in the tissues.

Two factors at least which might be responsible for the latter effect suggest themselves at once, namely, (*a*) shortage of oxygen arising from the increased oxygen requirements of the body, and (*b*) general rise of body temperature due to the muscular exertion.

In recent years the phenomena of deficiency of oxygen which may arise during muscular work have been ascribed, at least during severe exertion, to the action of lactic acid liberated in the active muscles in consequence of the metabolism of these muscles out-running the available oxygen supply that can be furnished by the blood stream,* and RYFFEL has, in fact, shown the presence of lactic acid in blood and urine as a result of hard muscular work.†

Lactic acid arising in this way will give rise to several effects. It will lower the alkalinity of the blood, and, since the activity of the respiratory centre is dependent on the hydrogen ion concentration in the blood, thus aid carbonic acid to excite the respiratory centre, a lower partial pressure of CO_2 being required to stimulate the centre to a given degree than would be the case in the absence of lactic acid. As lactic acid which has reached the blood stream is extremely slowly eliminated or destroyed (see Ryffel's data), it will tend to accumulate, and the hyperpnœa will, consequently, become much greater than one would expect from the CO_2 stimulus alone. If the excessive hyperpnœa caused in this way is sufficiently great, the alveolar CO_2 pressure may actually be reduced below the normal resting threshold value, in spite of the greatly increased production of CO_2 due to the muscular work, and directly this happens preformed CO_2 must be washed out of the body, the expulsion of this preformed CO_2 being unaccompanied by any corresponding increase in the oxygen absorption.

What is more, lowering of the alkalinity of the blood in severe muscular work is associated with a decrease in the absorption power of blood for CO_2,‡ and this decrease may be enormous with very severe work. A sudden diminution in the absorption power of blood for CO_2 must be accompanied by a great rise in the partial pressure of CO_2 in the blood, with, of course, an increase of the hydrogen ion concentration in correspondence, and consequently, if the muscular work is sufficiently severe to lead to the formation of lactic acid, the hyperpnœa must be still more exaggerated at the start until the redundant preformed CO_2 can be eliminated.

Were lactic acid to continue to accumulate, there is little doubt that the subject would find the work too severe for him, and he would soon be forced to stop; but

* DOUGLAS and HALDANE, 'Journ. Physiol.,' vol. 38, p. 420 (1909). See also DOUGLAS, HALDANE, HENDERSON, and SCHNEIDER, *loc. cit.*; and DOUGLAS, 'Ergebnisse der Physiol.,' 1914, p. 391.

† RYFFEL, 'Journ. Physiol.,' vol. 39, p. xxix, Proc. (1910).

‡ CHRISTIANSEN, DOUGLAS, and HALDANE, 'Journ. Physiol.,' vol. 48, p. 244 (1914); MORAWITZ and WALKER, 'Biochem. Zschr.,' vol. 60, p. 395 (1914).

there is some evidence to suggest that if lactic acid production is not very great and the work can be kept up for a considerable time, the rate of production of lactic acid will presently be balanced by the rate of its elimination or destruction, and a condition of equilibrium will be reached in which the threshold stimulating value of CO_2 on the respiratory centre will remain constant at some point which is below the normal and dependent on the steady amount of lactic acid present, and the ventilation of the lungs will now be determined, in the absence of other disturbing factors, by the height of the CO_2 pressure in the arterial blood above the new threshold stimulating value, not above the normal threshold value.

So long as expulsion of preformed CO_2 is occurring without corresponding increase in the absorption of oxygen, the respiratory quotient must be abnormally high, and muscular work of sufficient severity to give rise to lactic acid will therefore be characterisd both by excessive hyperpnœa and output of CO_2 and by an abnormal rise in the respiratory quotient. These changes will presumably be most marked at the commencement of the muscular exertion when the body is, so to speak, suddenly flooded with lactic acid, and there has not yet been time for any material reduction in the amount of preformed CO_2 in the body. Should, however, a balance be struck between production of lactic acid and its elimination, the abnormal hyperpnœa and respiratory quotient of the early stages will gradually diminish as the excess of preformed CO_2 is got rid of, till finally a condition of equilibrium will be reached in which the ventilation of the lungs will again become proportional to the mass of CO_2 given off, just as is the case in the absence of lactic acid or other disturbing factors, and the respiratory quotient will once more afford a true index of the character of the metabolism in the tissues. At this stage, even though the initial hyperpnœa has shut down to some extent, it of course remains in excess of what one would expect from the CO_2 stimulus alone, since there is also the constant action of lactic acid to be reckoned with.

If lactic acid is still present when the muscular work stops, the lowering of the threshold exciting value of CO_2 on the respiratory centre will become evident as the hyperpnœa dies away, since a considerable time elapses before the excess of lactic acid disappears, and a minute or two after the stop, the resting alveolar CO_2 pressure falls below the normal value. As the lactic acid disappears, a quantity of CO_2 must be retained to make up for the amount of preformed CO_2 originally expelled, and this will be accompanied by a gradual rise in the threshold stimulating value of CO_2 on the respiratory centre (*i.e.*, on the resting alveolar CO_2 pressure), as was shown by DOUGLAS and HALDANE, the normal threshold value being regained when the whole of the lactic acid has been eliminated. While CO_2 retention is occurring in this way, the respiratory quotient will be abnormally low, since there will be no corresponding reduction in oxygen consumption. If lactic acid production is limited only to the early period of the work, we should expect to find evidence of this

compensatory CO_2 retention at a later stage of the work, and possibly no indication of the presence of lactic acid when the work stops.

Lactic acid has so far only been definitely recognised in severe muscular work. It is, however, possible to imagine another method by which want of oxygen might exert its effect, and this might come into play with milder degrees of work. Thus supposing the arterial blood is not quite fully saturated with oxygen during the work, owing to the rate of passage of oxygen through the pulmonary epithelium being insufficient, and that this slight depression of the arterial oxygen pressure is in itself capable of exerting an effect on the respiratory centre, the threshold stimulating value of CO_2 on the respiratory centre will be lowered. Such an effect of want of oxygen will, however, presumably remain constant when the body has settled down to the work and be limited to the time during which the muscular work is actually in progress, for it should vanish with the reduction in the oxygen consumption on the cessation of the work. If this effect is sufficiently great to cause the alveolar CO_2 pressure to fall below the normal threshold value during the work, it should lead to temporary exaggerated hyperpnœa and expulsion of preformed CO_2 with an abnormally high respiratory quotient, just as does the accumulation of lactic acid. In this case, however, one would expect that apnœa would ensue when the oxygen want is relieved on the stop of the work and compensatory retention of CO_2 is taking place, for the threshold stimulating pressure of CO_2 on the respiratory centre should at once return to its old normal value, unless of course the deficiency of oxygen has led (in the absence of lactic acid) to some more lasting alteration of the threshold CO_2 pressure by a change in the "fixed alkalinity" of the blood, a condition which appears to come into force during prolonged exposure to atmospheres in which the oxygen pressure is reduced, *e.g.*, at high altitudes.

With a less degree of oxygen want of this type the effect should be of a different character. All that will happen will be that throughout the work the hyperpnœa will be somewhat greater, and the alveolar CO_2 pressure somewhat lower than would be expected if CO_2 alone afforded the stimulus to the respiratory centre; yet here again the correlation of the activity of the respiratory centre with the varying metabolism of the tissues will depend on CO_2, since the want of oxygen may be regarded merely as exerting a constant action during the work and causing the respiratory centre to respond to a greater degree than normal for a given rise of CO_2 pressure. So long as the alveolar CO_2 pressure during the work does not fall below the normal threshold value there will be no expulsion of preformed CO_2. Consequently, a determination of the total respiratory exchange ought under these circumstances to afford evidence neither of excessive output of CO_2 nor of temporary great exaggeration of the hyperpnœa, while the respiratory quotient should continue to afford a true indication of the character of the metabolism of the tissues. The effect of such a want of oxygen will only be rendered apparent during the work by studying the relationship of the hyperpnœa to the alveolar CO_2 pressure at the time.

A "direct" effect of want of oxygen on the respiratory centre of this nature might of course be superimposed on the effects due to accumulating lactic acid in the case of severe work.

In DOUGLAS'S case the behaviour of the CO_2 output and of the hyperpnœa do not appear to conform with what is required by the "lactic acid" or gross want of oxygen theories as stated above, for these values remain practically constant throughout the whole period of work, in spite of the slow steady rise in the oxygen consumption and corresponding fall in the respiratory quotient. No support is therefore given to the lactic acid hypothesis in this case.

In Experiments 3 and 6 we analysed the urine passed just before the experiment began, and that passed just after it terminated, for lactic acid by RYFFEL'S method,* and found in the second sample no more lactic acid than the trace found in the first. Our determinations of lactic acid were not made with the greatest care in these experiments, and the results given in Tables II–VI should be regarded as approximate: the trace of lactic acid found in the normal resting urine is referred to as negative in these Tables.

The actual identification of lactic acid in the urine is, however, probably too coarse a method to adopt if the production of lactic acid is small, for lactic acid might get destroyed in whole or part before it could be excreted by the kidneys, and RYFFEL shows in fact that after severe exercise, excess of lactic acid in the urine disappears more rapidly than does that in the blood.

Far the most delicate test for the lowering of the alkalinity of the blood owing to deficiency of oxygen or production of lactic acid is to be found in the reaction of the respiratory centre. DOUGLAS and HALDANE (*loc. cit.*) showed that if the alveolar CO_2 pressure is determined at intervals whilst resting after the stop of some severe muscular work, it shows a characteristic fall to a value much below normal, followed by a slow recovery, indicating that the threshold stimulating value of CO_2 on the respiratory centre has been temporarily lowered by the exercise. This reaction is, however, absent after gentle or moderate exercise, the threshold value after the exercise being the same as it was before.

We made three experiments on DOUGLAS by this method, using the HALDANE-PRIESTLEY method of obtaining alveolar air samples and employing the same degree of work as in the determinations of the respiratory exchange. The results are given in Table VIII.

The figures show that there was a definite, though small, temporary lowering of the alveolar CO_2 pressure after the exercise in the first only of these experiments, but it should be noted that the subject was not cooled by the fan in this experiment.

It is known that rise of body temperature in an otherwise normal person is associated with a degree of hyperpnœa out of proportion to the CO_2 production at

* 'Journ. Physiol.,' vol. 39, p. v, Proc. (1909).

TABLE VIII.

	Percentage of CO_2 in dry alveolar air.	Pressure of CO_2 in mm. Hg in alveolar air at 37° saturated with moisture.	Rectal temperature, degrees Fahrenheit.
(1) Work of 704 kg.m. per minute for 12 minutes.		No cooling by fan.	Bar. 765 mm.
Normal before start	5·58	40·0	
3½′ after stop	5·25	37·7	
12 ,,	5·29	38·0	
25½ ,,	5·23	37·6	
45 ,,	5·52	39·6	
63½ ,,	5·46	39·2	
(2) Work of 704 kg.m. per minute for 21 minutes. Bar. 764 mm.		Cooled by one fan during work.	
Normal before start	5·29	38·0	99·6
3½′ after stop	5·31	38·1	
11 ,,	5·14	36·9	
15 ,,	—	—	99·8
23½ ,,	5·39	38·7	
37 ,,	5·31	38·1	
52 ,,	5·32	38·2	
(3) Work of 704 kg.m. per minute for 20½ minutes. Bar. 742 mm.		Cooled by one fan during work.	
Normal before start	5·61	39·0	98·8
3½′ after stop	5·74	39·9	
10¾ ,,	5·68	39·5	
17 ,,	—	—	99·8
22 ,,	5·47	38·0	
34 ,,	—	—	99·2
37 ,,	5·58	38·8	

the time.* As HALDANE pointed out to us, the temperature which really matters in this connection is that of the blood reaching the respiratory centre. This, unfortunately, is practically impossible to estimate, since the blood coming from parts of the body which are abnormally warm owing to heightened metabolism is mixed in the heart with cooled blood coming from the skin. It is clear that a temporary rise in temperature of the blood reaching the respiratory centre during muscular work might give rise to alterations in the hyperpnœa, CO_2 output and respiratory quotient of the same sense as those caused by the development and disappearance of lactic acid. We recognised the difficulties of the question of temperature, but the best we could do was to try by means of fans to keep the general body temperature within reasonable limits during the work, for we were afraid that it might otherwise rise unduly in the still air of the laboratory. One fan was sufficient to keep the subject cool and comfortable in experiments at work of 704 kg.m. per minute, though two were necessary in

* HALDANE, 'Journ. of Hygiene,' vol. 5, p. 503 (1905); HILL and FLACK, 'Journ. Physiol.,' vol. 38, p. lvii, Proc. (1907).

the experiments with harder work. In each case the sweat evaporated almost as fast as it was formed, and the subject did not therefore get chilled in the long period of rest that followed the muscular exertion. From the data given in Table VIII, where a few determinations of the rectal temperature before and after the muscular work are recorded, we should judge that our efforts to prevent undue rise of temperature had been tolerably successful, though it is quite possible that in the first of these experiments the distinct fall in the alveolar CO_2 pressure after the work is dependent more on rise of temperature than on anything else, as no fan was used.

The lactic acid theory and temperature changes therefore appear inadequate to explain the rise of the respiratory quotient during the work in DOUGLAS'S case in this series of experiments, and, as we have pointed out above, a slight "direct" action of deficiency of oxygen on the respiratory centre, if limited to the period of work, would not be appreciable in a record of the total respiratory exchange, though it might become evident from a consideration of the volume of air breathed during the work and the prevailing alveolar CO_2 pressure. We suggest, therefore, that this alteration is, in the main at least, due to the fact that the energy output during the work involves the metabolism of a greater proportion of carbohydrate to fat than is the case during rest.

This idea is strengthened by the earlier experiments on DOUGLAS during walking exercise,* in which it was shown that rise of respiratory quotient could be detected at rates of exercise so low as to demand only double the resting metabolism, though the rise became greater as the rate of walking and the total metabolism increased, and that moderate prolongation of the exercise did not lead to any striking alteration of the respiratory quotient. It was tentatively suggested on these grounds, as well as on the fact that examination of the alveolar air gave no indication of a persistent lowered threshold value of CO_2 unless the pace of walking was very fast, that the most probable explanation lay in the increased proportion of carbohydrate to fat consumed during the work. AMAR,† using a bicycle ergometer, had previously obtained very similar results and had reached the same conclusion. A number of BENEDICT and CATHCART'S experiments show a fairly considerable rise of respiratory quotient during the work, with in some cases a tendency to diminution with prolongation of the exercise, though in a good many instances the respiratory quotient remains just about the same during the work as it was during the preliminary resting period, as was the case with HOBSON in our experiments. The average of their experiments shows that there is a distinct, though slight, rise of respiratory quotient during the work, and in a full discussion of the significance of this change they incline strongly to the view that it indicates an increase in the proportion of carbohydrate consumed. BENEDICT and MURSCHHAUSER'S experiments on trained athletes during walking exercise afford many examples of a rise of respiratory quotient during the exercise, and

* DOUGLAS, HALDANE, HENDERSON, and SCHNEIDER, *loc. cit.*

† AMAR, 'Le Rendement de la Machine Humaine,' Paris, 1910.

though this change is most marked when the subject had had food shortly before the commencement of the experiment, it also occurs to a less degree when he was in the post-absorptive state. The diminution of respiratory quotient during long periods of exercise is also shown in these experiments. Apparently, therefore, our experiments are not vitiated by the fact that the subjects had had breakfast about two hours before the commencement, though no doubt the degree of alteration that we observed was exaggerated by the fact that the subjects were not in the post-absorptive state.

If the view is right that carbohydrate may be used in greater proportion during work than during rest, a diminution of respiratory quotient during the work would seem by no means improbable, since the degree of rise will no doubt be to some extent dependent on the availability of carbohydrate, and this will quite likely be lessened as the stores of carbohydrate in the muscles or in the body at large are depleted in consequence of the heightened metabolism. It is clear from all these experiments that there is a good deal of difference between different individuals, and between the same individual at different times, as regards the character of their metabolism. Some, like HOBSON, seem to have much the same type of metabolism during work as during rest, at least under circumstances when the work is not very hard, while others like DOUGLAS appear to consume an increased proportion of carbohydrate, as indicated by a definite rise of respiratory quotient, even when the work is quite light. It is, of course, possible that the degree of physical fitness for the work in question may be of importance in this connection.

It is of interest to consider whether it is possible in these experiments to explain the hyperpnœa merely by a rise of CO_2 pressure in the arterial blood along the lines originally suggested by HALDANE and PRIESTLEY.* KROGH and LINDHARD† have raised a valid objection to direct determinations by the HALDANE–PRIESTLEY method of the alveolar CO_2 pressure (with which the arterial blood must be nearly in equilibrium) during muscular work, and one must therefore use an indirect method, though the direct method is applicable during rest.

The average normal alveolar CO_2 percentage during rest was about 5·5 per cent. in DOUGLAS (Tables VIII and IX). Taking an average of all the observations during the period of rest before commencing the work (Tables II to VI), the volume of each breath was 703 c.c. and the CO_2 percentage in the expired air 3·6 per cent. Calculation from these figures gives an effective dead space at rest of 244 c.c. (this includes the dead space of 60 c.c. in the valves and mouthpiece). An average of all the observations on DOUGLAS during work of 704 kg.m. per minute shows a volume for each breath of 1974 c.c. and an expired CO_2 percentage of 4·83 per cent. If we assume that the dead space during the hyperpnœa caused by the work has the same value as during rest, the calculated alveolar CO_2 percentage during the work is

* HALDANE and PRIESTLEY, 'Journ. Physiol.,' vol. 32, p. 225 (1905).

† KROGH and LINDHARD, 'Journ. Physiol.,' vol. 47, p. 30 (1913); *ibid.*, p. 431.

5·51 per cent., *i.e.*, a value identical with the resting normal. Any increase in the dead space during the work above the normal resting value would, of course, imply an increase in the alveolar CO_2 percentage.

We have previously shown* that the effective dead space shows a considerable increase during the hyperpnœa, caused by breathing air containing CO_2 when the oxygen consumption and CO_2 production are practically the same as during rest, and HALDANE† and YANDELL HENDERSON, CHILLINGWORTH and WHITNEY,‡ have shown that the effective dead space increases rapidly with the depth of the breaths in experiments in which the depth of the breathing was altered voluntarily. KROGH and LINDHARD,§ on the contrary, maintain that the effective dead space is hardly altered at all, no matter whether the breathing is deep or shallow. While it seems to us possible that the method of investigation adopted by KROGH and LINDHARD may give information regarding the volume of air contained in the respiratory passages, including trachea, bronchi and bronchioles, and its variations, we are not convinced by their argument that this anatomical dead space is identical with the true effective dead space, which is a purely conventional though very convenient expression, by which we assume the expired air to be composed of a mixture of average "alveolar air" with pure air contained in the "dead space."

Our previous experiments on DOUGLAS have shown that, during a hyperpnœa, caused by breathing air containing CO_2, a rise of 0·28 per cent. in the alveolar CO_2 percentage at normal barometric pressure (*i.e.*, of 2 mm. pressure of CO_2) is sufficient to cause a rise of 10 litres in the total ventilation of the lungs. If we suppose in our present experiments that, during work of 704 kg.m. per minute, the threshold stimulating value of CO_2 on the respiratory centre remained the same as during rest, corresponding to 5·5 per cent. of CO_2 in the alveolar air, the average rise during the hyperpnœa of about 30 litres above the normal total ventilation of the lungs at rest would have required a rise of about 0·84 per cent. above the resting alveolar CO_2 percentage to account for it. With the average volume of each breath at 1974 c.c., and expired CO_2 percentage of 4·83 per cent., the required percentage of CO_2 in the alveolar air (viz., 6·34 per cent.) would have been obtained if the effective dead space (including valves) had been 472 c.c. So far as we can judge from the experiments by ourselves, by HALDANE, and by HENDERSON and his colleagues, this value for the dead space is quite reasonable, having regard to the depth of the breathing. At the same time, it should be remembered that the rise of alveolar CO_2 pressure, that we should otherwise expect to find to account for the hyperpnœa, would be proportionally lowered if there were any "direct" action of slight deficiency of oxygen on the respiratory centre during the period of muscular work.

* CAMPBELL, DOUGLAS, and HOBSON, 'Journ. Physiol.,' vol. 48, p. 303 (1914).

† 'Amer. Journ. Physiol.,' vol. 38, p. 20 (1915).

‡ 'Amer. Journ. Physiol.,' vol. 38, p. 1 (1915).

§ 'Journ. Physiol.,' vol. 51, p. 59 (1917).

In the case of HOBSON, we have only two earlier determinations of the normal alveolar CO_2 percentage at rest,* and these give an average value of 5·2 per cent. If we accept this value, his resting dead space in our experiments was about 205 c.c., and, if this had remained unchanged during the muscular work, his alveolar CO_2 percentage during the work would have been about 5·6 per cent., *i.e.*, above the resting value.

(ii) *Work of* 1056 *kg.m. per Minute.*

Experiments 8 and 9 on DOUGLAS, and Experiment 10 on HOBSON, show a quite abnormal rise of the respiratory quotient to unity or beyond in the early period of the work. This large rise is, however, quite shortlived, for, by the time the second observation is made, the respiratory quotient has fallen to about 0·95 in DOUGLAS'S case and to 0·88 in HOBSON'S. After the temporary sharp rise of the respiratory quotient has passed away, the picture presented for the remainder of the period of muscular work is very similar to that shown in the experiments at 704 kg.m. per minute, *i.e.*, DOUGLAS'S respiratory quotient remains at a level considerably above the resting value, with a tendency to fall as the work is prolonged and the oxygen consumption rises, while HOBSON'S returns to about its preliminary resting value. It will be noted in Experiments 9 and 10 that the CO_2 output during the first work period is markedly in excess of what it is subsequently, and that the hyperpnœa is definitely at a maximum during the same period. In the later work periods, both CO_2 output and hyperpnœa have dropped back to fairly steady values.

These changes shown in the early period of the exercise can be explained quite simply on the "lactic acid" hypothesis, coupled, it may be, with a more direct action of want of oxygen on the respiratory centre, for there is clear evidence of excessive hyperpnœa and washing out of pre-formed CO_2 at this time. Moreover, 0·1 grm. of lactic acid was recovered from the urine passed at the end of Experiment 8, and 0·05 grm. at the end of Experiment 9.

In addition to this, we obtained definite evidence in DOUGLAS' case of a persistent, though not very great, lowering of the threshold stimulating value of CO_2 on the respiratory centre by following the alveolar air changes after the cessation of work at 1056 kg.m. per minute. The figures are given in Table IX.

It will be seen that the resting alveolar CO_2 percentage fell from the normal of about 5·5 per cent. to about 5·1 per cent. between roughly the fourth and twentieth minutes after the stop (*i.e.*, a fall of about 2½ mm. below the normal alveolar CO_2 pressure). The general rise of body temperature seems in this instance again to have been fairly effectively controlled by the arrangements we adopted for cooling the subject.

The shortness of the period during which the abnormally high respiratory quotient and excessive hyperpnœa are shown in these experiments, and the slightness of the

* CAMPBELL, DOUGLAS, HALDANE, and HOBSON, 'Journ. Physiol.,' vol. 46, p. 301 (1913).

TABLE IX.

	Percentage of CO_2 in dry alveolar air.	Pressure of CO_2 in mm. Hg in alveolar air at 37° saturated with moisture.	Rectal temperature, degrees Fahrenheit.
(1) Work of 1056 kg.m. per minute for 21 minutes. Bar. 757 mm.		Cooled by two fans during work.	
Normal before start	5·37	38·2	99·5
4′ after stop	5·23	37·2	
11 ,,	5·23	37·2	
18 ,,	—	—	100·3
22¾ ,,	5·13	36·4	
31½ ,,	5·28	37·5	
47 ,,	5·33	37·8	
58 ,,	—	—	98·9
61½ ,,	5·50	39·1	
71 ,,	—	—	98·7
77½ ,,	5·46	38·8	
(2) Work of 1056 kg.m. per minute for 20 minutes. Bar. 761 mm.		Cooled by two fans during work.	
Normal before start	5·52	39·4	99·2
4¾′ after stop	4·87	34·8	
11¾ ,,	5·07	36·2	
18 ,,	—	—	99·9
21¾ ,,	5·30	37·8	
32 ,,	—	—	99·4
35¾ ,,	5·24	37·4	
46 ,,	—	—	99·0
50 ,,	5·48	39·1	

after-effect on the alveolar CO_2 pressure, may tempt one at first sight to suggest that the lactic acid was mainly produced in the early period of the exercise, at a time, perhaps, when the circulation had not yet become fully adapted to meet the demand made on it owing to the sudden increase in the metabolism of the active muscles. This may be so, but it will be evident from what we have said previously that the figures as they stand do not justify this interpretation, for we might get a result of the same character if lactic acid were produced at a slow rate throughout the muscular work and elimination or destruction of lactic acid were gradually to balance its production. Under these circumstances, as the initial disturbance caused by the production of lactic acid dies away, a change in the respiratory quotient, due to an alteration in the character of the metabolism in the tissues, should gradually be unmasked, and, in the later periods of work in these experiments, any persistent increase of the respiratory quotient may well have the same explanation as the one we have already put forward in the case of lighter work, viz., an increase in the proportion of carbohydrate to fat metabolised. In order to settle the point clearly, the simplest plan would appear to be to eliminate so far as practicable any possibility of want of oxygen, and this could be done by working in an atmosphere containing a

higher percentage of oxygen than normal. Unfortunately, we have not yet had the opportunity of making these experiments.

Experiment 11 on HOBSON differs from the other experiments with this degree of work, as it resembles closely the result we got on DOUGLAS with work of 704 kg.m. per minute. The work leads to a rise of respiratory quotient, which diminishes progressively during the course of the work, but there is no definite evidence pointing to excessive hyperpnœa and washing out of pre-formed CO_2 in the early stages. Only a trace of lactic acid could be found in the urine at the end of the experiment, and this was probably not in excess of what is shown by normal urine. Apparently, therefore, HOBSON was not affected by any gross want of oxygen in this experiment, though, in Experiment 10, the additional demand of 200 c.c. of oxygen per minute, entailed by the disadvantageous conditions under which he was working, sufficed to put him in difficulties from this cause. Owing to HOBSON's better physical condition, one would naturally expect him not to show want of oxygen effects as soon as DOUGLAS.

If we discard in the experiments on DOUGLAS the early periods in which the respiratory quotients are clearly abnormally high, and confine ourselves to the last observation during work in Experiment 8, and the last three in Experiment 9, the average volume of each breath during the work is 2451 c.c., and the CO_2 percentage in the expired air 4·52 per cent. Assuming the resting effective dead space of 244 c.c. to hold good during the work, the calculated alveolar CO_2 percentage during the work becomes 5·01 per cent., a value which is considerably below the normal found at rest, but approximately the same as the temporary low value found shortly after the cessation of the work (Table IX). The average total ventilation of the lungs is 60·5 litres per minute, *i.e.*, about 52·5 litres above the resting value. In the case of hyperpnœa caused by breathing air containing CO_2, this increase would have been caused in DOUGLAS'S case by a rise of 1·47 per cent. above the normal alveolar CO_2 percentage. If we assume in the experiments on DOUGLAS, with work of 1056 kg.m. per minute, that the hyperpnœa, when it has steadied down after the preliminary disturbance due to lactic acid production, is proportional to the mass of CO_2 produced, and the respiratory centre reacts to the same degree to changes of CO_2 pressure as during rest, we should expect to find an average rise in the alveolar CO_2 percentage of 1·47 per cent., not above the resting normal value of 5·5 per cent., but above the lowered threshold value maintained during the work owing to the effects of want of oxygen. The only index of this latter figure is furnished by the low value of about 5·1 per cent., to which the alveolar CO_2 falls after the stop of the exercise, though very likely this is not so low as that which prevailed during the work. Supposing the real average alveolar CO_2 percentage during the work had been 5·1+1·47, viz., 6·57 per cent., the effective dead space calculated from this figure, the average volume of a breath, and the average CO_2 percentage in the expired air would become 761 c.c., a large figure admittedly,

but still by no means out of the way in comparison with the observations of ourselves, of HALDANE, and of HENDERSON and his colleagues, which we have quoted previously. Here, again, the anticipated rise of alveolar CO_2 pressure and the calculated volume of the dead space would have been considerably reduced during the work if a "direct" effect of oxygen deficiency on the respiratory centre had been superimposed upon the effects caused by lactic acid accumulation.

In HOBSON'S case, if we assume the resting dead space to remain constant during the work, his alveolar CO_2 percentage during the work (Experiments 10 and 11) would be about 5·48 per cent., *i.e.*, a little above his resting normal and a little below the alveolar CO_2 percentage calculated during the work of 704 kg.m. per minute.

(iii) *Work of* 1232 *kg.m. per Minute.*

The results obtained in the single determination made during the work are evidently an exaggeration of those observed in the earlier period of work in Experiments 8, 9, and 10. No less than 0·57 grm. of lactic acid was recovered from the urine passed at the end of the experiment. Though the hyperpnœa was still very excessive, the respiratory quotient was only 1·03 when it was determined just before the stop, but it is evident that it must have had a much higher value at an earlier stage of the work before a great part of the excess of preformed CO_2 had been blown off. It is most unfortunate that we were prevented from determining the respiratory exchange at the end of the first 4 minutes of the work, but we can get some idea of what might have been happening in the earlier stages from some previously unpublished observations of DOUGLAS, HALDANE, and BOOTHBY. These are shown in Table X.

TABLE X.

Body weight in lbs.	Pace in miles per hour.	Kg.m. of lifting work per minute.	Respiratory exchange in c.c. per minute at 0° and 760 mm.		Respiratory quotient.	Breaths per minute.	At 37°, moist, and prevailing barometer.		CO_2 per cent. in expired air.
			O_2.	CO_2.			Litres breathed per minute.	C.c. per breath.	
DOUGLAS.									
150	2·8	1132	2870	3490	1·22	41·4	109·4	2670	3·95
—	2·7	1095	2940	3420	1·16	45·5	104·8	2300	4·04
HALDANE.									
183	2·5	1125	2790	3015	1·08	39·0	80·3	2060	4·64
BOOTHBY.									
113	1·6	570	2750	3300	1·20	39·0	96·1	2465	4·25

The work in this instance consisted in pushing a motor bicycle weighing about 150 lb. up an average gradient of 10·8 per cent., and the muscular work was continued for three minutes only before the sample of expired air was collected in a bag carried on the back of the subject, the collection of the sample taking rather over one minute. The walk was begun at a point where the gradient was far less steep and about one minute elapsed before the gradient of 10·8 per cent. was reached. The second experiment on DOUGLAS was made 1½ hours after the first. BOOTHBY started walking at too fast a pace to keep it up, and as he was slowing up very rapidly towards the end of the period of sampling owing to exhaustion, the average pace of 1·6 miles an hour does not give a real indication of the rate of work that he really reached during the observation.

These experiments were originally made simply to get some idea as to how far the respiratory exchange could be increased in really severe muscular work. The subjects were absolutely untrained, but BENEDICT and CATHCART'S observations show that a trained athlete can keep up work entailing an oxygen consumption of 3000 c.c. per minute, or even more, for some time. Judging by the oxygen consumption in DOUGLAS'S case the metabolism was perhaps a little higher than in Experiment 12, but the hyperpnœa and distress seemed to be much the same in the two cases. Respiratory quotients as high as 1·2 were reached, the volume of CO_2 produced per minute being in excess of the volume of oxygen absorbed by amounts varying in the different experiments from 225 c.c. in HALDANE'S case to 620 c.c. in the first observation on DOUGLAS. These figures give some idea what an enormous volume of preformed CO_2 may be expelled in very severe work. The excessive hyperpnœa and the lowness of the CO_2 percentage in the expired air are particularly marked in the experiments on DOUGLAS and BOOTHBY.

Further evidence regarding the cause of the very violent hyperpnœa, and the amount of preformed CO_2 that may have to be got rid of in short experiments when the work is extremely severe, is afforded by the observations of CHRISTIANSEN, DOUGLAS, and HALDANE (*loc. cit.*), in which it was shown that muscular work of this character led to an enormous temporary alteration in the CO_2 absorption power of the blood, the volume of CO_2 that can be held in combination in the blood at any given partial pressure of CO_2 being greatly reduced, as well as to a very great lowering of the threshold stimulating value of CO_2 on the respiratory centre, recovery of the normal absorption curve and normal threshold value of CO_2 not being attained till an hour or more after the stop of the muscular work.

(3) *The Respiratory Exchange during the Period of Rest following the Muscular Work.*

One of the most striking features in our experiments is the exceedingly rapid fall of both the respiratory exchange and the hyperpnœa as soon as the muscular exercise

stops. It has, of course, long been recognised experimentally* that such a fall occurs, though it is not till one can plot out the actual values as a continuous record that one fully appreciates its character. It is quite clear from figs. 4–7 that by far the greater part of this fall occurs in the first minute or two after the stop of the work, though a number more minutes elapse before the respiratory exchange and the hypernœa definitely fall to a steady value. The total period elapsing after the stop of the exercise before the respiratory exchange reaches a steady minimum varies in different experiments from 11 to 28½ minutes, with an average of about 20 minutes. We may presumably regard these final steady values as representing truly the more or less lasting effects produced on the resting respiratory exchange by a period of muscular work, and it seems reasonable to look upon the rapidly diminishing respiratory exchange in the period between the stop of the work and the assumption of these steady values as being determined, both in amount and character, by certain temporary causes incidental to the transition from work to rest.

The respiratory exchange after the stop of the muscular work is excessive for a short time in comparison with the figure at which it remains constant in the later stages, and it is of interest to ascertain the magnitude of this "excess respiratory exchange." This can be done in the case of some of our experiments in which the data are sufficiently complete by calculating the total respiratory exchange for the whole period from the stop of the work till the respiratory exchange reaches a reasonably steady value, and subtracting from the figure so obtained the total respiratory exchange calculated for the same period at the final steady rate. The figures are given in Table XI.

By comparison with Tables II–VI it will be seen that the "excess oxygen consumption" after work of 704 and 1056 kg.m. per minute on the average barely amounts to as much as the oxygen consumption during one minute of the muscular work, while the "excess CO_2 production" is considerably higher than the CO_2 production during one minute of the muscular work. In the case of the severe work of 1232 kg.m. per minute the "excess respiratory exchange" is greatly above the respiratory exchange during one minute of the previous muscular work. The values of this "excess respiratory exchange" after the work of 704 and 1056 kg.m. per minute are surprisingly low, especially as regards the oxygen consumption, but agree completely with the conclusions of earlier observers. LOEWY, for instance, states† that after the cessation of muscular work the respiratory exchange remains high for several minutes, though the excess oxygen consumption during the entire period of rest following the exercise scarcely amounts to so much as the oxygen consumption

* SPECK, 'Physiologie d. menschl. Atmens,' p. 56, Leipzig (1892); KATZENSTEIN, 'Pflügers Archiv,' vol. 49, p. 330 (1891); LOEWY, 'Pflügers Archiv,' vol. 49, p. 405 (1891); ZUNTZ und SCHUMBURG, *loc. cit.*, pp. 223, 235; ZUNTZ und HAGEMANN, 'Stoffwechsel des Pferdes,' p. 286, Berlin (1898).

† *Loc. cit.* See also LOEWY, 'Oppenheimer's Handbuch der Biochemie,' vol. 4, Part I, p. 262, Jena (1911).

during a single minute of the work period, and only mounts to a higher value when there is great muscular fatigue or when the work is done under conditions of insufficient supply of oxygen.

TABLE XI.

Experiment.	Work in kg.m. per minute.	Final period after the work in which the respiratory exchange is steady.			Period intervening between the stop of the work and the assumption of the steady respiratory exchange.		
		Length of period.	Average respiratory exchange in c.c. per minute.		Length of period.	Total O_2 consumption and CO_2 production in c.c. in excess of values which would have been obtained if the respiratory exchange during this period has been the same as the steady rate eventually reached.	
			O_2.	CO_2.		O_2.	CO_2.
DOUGLAS.							
2	704	33′ 37″	297	230	21′ 2″	1775	2262
3	704	33 31	298	234	18 3	1598	1807
8	1056	25 6	287	222	17 58	2512	3543
9	1056	68 37	328	248	10 56	1880	2720
12	1232	57 4	308	204	28 27	3708	5981
HOBSON.							
4	704	21 55	342	300	20 35	1363	1650
11	1056	32 55	350	285	24 30	2068	2498

Several different factors must combine to prevent the respiratory exchange from falling to a steady resting value immediately after the work stops, and some of these suggest themselves at once.*

(i) At the moment when the muscular work stops, blood, whose gaseous content has been determined by the last few moments of muscular activity, is in process of passing from the active muscles to the lungs. Clearly there can be no material decrease in the gaseous exchange in the lungs till the last of this blood has passed through the lungs.

(ii) It is improbable that at any given moment in the course of muscular activity the gaseous content of the blood leaving the active muscles corresponds with the actual consumption of oxygen or formation of CO_2 at the same moment in these muscles. The changes in the blood must lag a little behind the changes in the muscles, partly on account of the time required for the passage of gases between the blood and the muscles, and partly perhaps on account of the nature of the processes involved in the absorption of oxygen and production of CO_2 in a muscle.† At the

* In this connection see DURIG und ZUNTZ, 'Skand. Archiv für Physiol.,' vol. 29, p. 133 (1913).

† See FLETCHER and HOPKINS, 'Roy. Soc. Proc.,' B, vol. 89, p. 444.

same time it would appear that a lag of this description must be a pretty small thing under normal conditions of activity with an effective circulation of the blood through the muscles, otherwise it is difficult to see why it would not act prejudicially if the work were to be kept up for more than a short time, though the lag will be somewhat accentuated in the case of CO_2 by the capacity of the blood and tissue fluids for absorbing more CO_2 at higher partial pressures of the gas.

(iii) It is possible that complete rest is not assumed at once on stopping the work, or the "tone" of the muscles may only decrease gradually to a steady value after a period of activity. When determining the resting respiratory exchange it is always necessary to wait for some time before a reasonably steady value can be obtained, even if the preceding activity has been quite slight.

(iv) General rise of body temperature causes an increase of the resting metabolism.* When, therefore, rest is resumed after a period of muscular activity one would expect the metabolism in those parts of the body in which the exercise has caused a rise of temperature to remain unduly high until the temperature falls back to its normal value.

(v) The hyperpnœa after the stop of the muscular exercise decreases in much the same manner as does the respiratory exchange, keeping pace with CO_2 production rather than with oxygen consumption. So long as the hyperpnœa persists it implies some increase in the resting metabolism on account of the heightened activity of the respiratory muscles.

Of these factors, the first, which would have a considerable effect, as well as the second, one would expect to be limited to a brief period after the stop of the work, whereas the last three would be operative for a longer period and would cease more gradually. The excess respiratory exchange per minute in our experiments is far greater in the first period after the stop than in any of the subsequent periods, and after the great primary fall diminishes more and more gradually as the final steady value of the respiratory exchange is approached.

The fact that there is a temporary sharp rise of the respiratory quotient to 1·0 or beyond, *i.e.*, that the CO_2 output is for a brief time actually in excess of the oxygen absorption, immediately after the stop of the exercise has been noticed by other observers (Loewy, Katzenstein, Zuntz and Hagemann, Zuntz and Schumburg; *loc. cit.*). These authors have suggested that the cause of this is to be found in the fact that in addition to CO_2 other substances derived from the active muscles (*e.g.*, lactic acid) stimulate the respiratory centre, and that on cessation of the work the breathing remains exaggerated until these substances can be oxidised, the exaggeration of the breathing implying, of course, washing out of CO_2 from the body in excess of the oxygen absorbed.

This theory alone seems to us quite insufficient to explain the facts. The sharp rise of the respiratory quotient is shown in our experiments no matter whether the

* Sutton, 'Journ. Path. and Bact.,' vol. 13, p. 62 (1909).

work is light or heavy, or the period of exercise short or long, though the rise is more marked in the experiments with work of 1056 kg.m. per minute than in those with work of 704 kg.m. per minute. We have shown above that we could get no distinct evidence of the formation of lactic acid in the case of work of 704 kg.m. per minute. In the experiments with work of 1056 kg.m. per minute we got unmistakable evidence of the formation of lactic acid in several cases; the amount, however, did not appear to be very great, while it seemed quite likely that in the later stages of the work production and elimination of lactic acid were balanced. In neither of these cases does there seem to be any reason for supposing that CO_2 would be expelled from the body after the stop of the work out of proportion to the oxygen consumed.

The facts seem to us capable of another interpretation. Whilst admitting that there will no doubt be a tendency during the work to accumulation of CO_2 in the active parts of the body owing to the amount of CO_2 held in the blood and tissue fluids being dependent on the partial pressure of CO_2, and that this excess will be given off directly the work stops, the close similarity both in general character and in rate between the diminution of the hyperpnœa after stopping the work and the diminution of the hyperpnœa when breathing is resumed after holding the breath,* or when fresh air is breathed again after exposure to an atmosphere containing CO_2†, suggests the necessity of a deeper explanation. In the latter two cases the difficulty was pointed out of explaining the gradual subsidence of the hyperpnœa without any signs of marked periodicity of the breathing, unless the assumption was made that the respiratory centre is itself charged up with more CO_2 than normal in consequence of the rise of alveolar CO_2 pressure while the breath is held or air containing CO_2 is breathed, and that the washing out of this excess from the respiratory centre when the breathing recommences or fresh air is again breathed takes an appreciable time.

It appears to us probable, therefore, that the alveolar CO_2 pressure during the work is above the resting threshold stimulating value which holds good just after the exercise stops—this may be the same as the normal threshold value in the case of light work, or lower than the normal in the case of heavy work owing to the effect of lactic acid (and perhaps rise of temperature), and that CO_2 is therefore dammed back in the body at large, including the respiratory centre, during the work. It has been shown above that this condition can only be attained in our experiments if the effective physiological dead space during the hyperpnœa is increased during the work above the normal resting value. Directly the muscular work ceases the alveolar CO_2 pressure will tend to fall as CO_2 production is reduced, and the CO_2 which was dammed back in the body will be expelled, the expulsion being rapid at first while the excess of CO_2 retained in the body is still high, and then becoming slower and slower as the amount of CO_2 retained sinks towards the value corresponding to the prevailing threshold CO_2 pressure. Expulsion of CO_2 dammed back in this manner

* DOUGLAS and HALDANE, *loc. cit.*

† CAMPBELL, DOUGLAS, HALDANE, and HOBSON, *loc. cit.*

would not be accompanied by any corresponding variation in the oxygen consumption. One would expect that the higher CO_2 production in the heavier work experiments would be accompanied by a higher relative increase in the alveolar CO_2 pressure than is the case with the lighter degree of work, and this will help to explain why the sharp rise of the respiratory quotient after the stop of the work is more pronounced with work of 1056 kg.m. per minute than it is with work of 704 kg.m. per minute.

If, however, the body has not attained equilibrium with the lactic acid production by the time the work stops, it is evident that the action of lactic acid must continue during the earlier period of rest following on the muscular exercise, and that its effect will be superimposed on that postulated above. This condition must be present in Experiment 12 at least, for as the respiratory quotient was still above unity in the last period of work it is clear that the expulsion of preformed CO_2 was not by any means complete when the work stopped, and this process must have continued for some time after resuming rest.

The precise height to which the respiratory exchange will rise after the exercise stops will depend in observations of this type on the length of the period of observation, for the longer the period the lower must be the apparent rise. At the same time one would expect the rise to be most evident not in the first few moments after the stop of the work, when the gaseous content of the blood reaching the lungs is still largely determined by the gaseous exchange of the muscles in the final stage of of the work, but at a slightly later period when the respiratory exchange has dropped considerably. This seems to be the case in Experiment 12, where the highest respiratory quotient was observed not in the first 51 seconds after the subject resumed rest, but in the succeeding period of 1 min. 37 secs.

It would be of very great interest to ascertain the amount of surplus CO_2 which is expelled without corresponding intake of oxygen in the early period after the stop of the exercise, but our data are insufficient for this purpose, though it is possible to get some idea as to the order of magnitude of this quantity. It would no doubt be right to regard the volume of CO_2 produced in excess of the total volume of oxygen absorbed whilst the respiratory quotient is above unity as derived from CO_2 dammed back or preformed in the body, and to take this as the minimal value. During this time, as well as in the subsequent two or three periods, there is, however, nothing to indicate the true respiratory quotient significant of the character of the metabolism in the tissues as distinct from the observed respiratory quotient, which must differ from the former in a degree depending on the amount of surplus or preformed CO_2 expelled or on the amount of CO_2 retained to make up for a deficiency previously created. It would seem to us that a still fairer approximation would be obtained by taking the whole period following the stop of the muscular work in which the respiratory quotient is above the value found in the last work period, and reckoning as surplus CO_2 expelled from the body the volume of CO_2 in excess of what is required by the oxygen intake if one allows a respiratory quotient identical with that shown in the

last work period. The real metabolic respiratory quotient directly after the work can hardly exceed that shown during the last stages of the work; in fact, the general course of events would suggest that it begins to fall very soon after the exercise stops. We can obtain a limiting value for this surplus CO_2 discharge by assuming that the real metabolic respiratory quotient falls immediately after the stop of the work to its final steady value, and calculating the excess CO_2 on this assumption for the whole period which elapses before the respiratory exchange and observed respiratory quotient become steady, for such a value must be in excess of the true one since the respiratory exchange and metabolic respiratory quotient are bound to approximate in the first few moments after the stop to the values shown during the last stages of the work, while no account is taken of the possibility that the fall of the respiratory quotient may be in part attributable to retention of CO_2. The values calculated by these three methods are given in Table XII, and it will be seen that with the exception of Experiment 11 the apparent surplus of CO_2 expelled is greater after the heavier work than after the lighter.

TABLE XII.

Experiment.	Work in kg.m. per minute.	Surplus CO_2 production after the stop of the work in c.c., reckoned on the assumption that it indicated so long as the respiratory quotient is above :—		
		1·00	Value shown in last period of work.	Final steady value shown in later periods of rest.
DOUGLAS.				
2	704	114	—	882
3	704	132	322	557
5	704	20	354	—
6	704	—	253	—
8	1056	540	678	1598
9	1056	380	570	1290
12	1232	620	—	3531
HOBSON.				
4	704	106	296	445
7	704	63	287	—
10	1056	272	620	—
11	1056	90	302	818

One slight source of error in our results may be alluded to here, since it is likely to be most evident just after the work stops. The bag method can only give a perfectly correct result provided that the composition of the air left in the lungs at the end of the period of sampling is identical with that at the beginning. This condition is fulfilled in the normal resting condition or when the subject is in equilibrium with the work. If, however, the composition of the air in the lungs alters while the expired

air is collected an error is introduced in the calculation of the CO_2 output and oxygen absorption. Thus if the CO_2 percentage in the air in the lungs is lower at the end of the period than it was at the beginning, a change which is likely to occur when rest is resumed after a period of work, the contents of the bag will indicate a rather higher CO_2 production and oxygen consumption than the true respiratory exchange of the body at the time. Our periods of sampling were, however, so long, and the total CO_2 production and oxygen consumption during the period so large, that any error due to this cause becomes negligible, the more so as the chest is in the expiratory position when the period begins and when it ends, and the volume of air contained in it is therefore small. Change of respiratory quotient in the air in the lungs might also cause a slight error in the observed respiratory quotient, but having regard to the general rate at which the respiratory quotient alters in our experiments this error would be quite trivial.

It will be seen in Tables II and III that the respiratory exchange and respiratory quotient eventually fall back after the stop of the work of 704 kg.m. per minute to just about the same values as were shown during the preliminary rest (see also Table XIII below). In DOUGLAS's case there is some evidence that the respiratory quotient falls to a minimal value, which is slightly below the original resting value, about 20–30 minutes after the stop of the exercise, and may then rise again to the

TABLE XIII.

Experiment.	Work in kg.m. per minute.	Preliminary resting period.			Final steady period after the work.		
		Respiratory exchange in c.c. per minute.		Respiratory quotient.	Respiratory exchange in c.c. per minute.		Respiratory quotient.
		O_2.	CO_2.		O_2.	CO_2.	
DOUGLAS.							
1	704	286	227	0·793	—	—	—
2	704	—	—	—	297	230	0·775
3	704	—	—	—	298	234	0·785
5	704	294	239	0·813	—	—	—
		293	231	0·788			
6	704	263	213	0·808	269	212	0·788
8	1056	281	233	0·829	287	222	0·773
9	1056	308	266	0·863	328	248	0·756
12	1232	—	—	—	308	204	0·662
HOBSON.							
4	704	385	331	0·860	342	300	0·877
		350	308	0·880			
7	704	—	—	0·866	—	—	—
10	1056	394	349	0·885	—	—	—
		423	373	0·881			
11	1056	375	323	0·860	350	285	0·815

preliminary resting value. This apparent variation in the respiratory quotient depends, however, on such small differences in the observed values of CO_2 output and oxygen consumption that it becomes rather dubious. There is certainly no marked diminution of the respiratory quotient which we should have expected if the rise of the respiratory quotient during the work had been due to expulsion of preformed CO_2, and had been followed after the stop of the work by retention of a corresponding amount of CO_2. The absence of this feature supports the view, therefore, that the rise of the respiratory quotient during the work is in this case due to a relatively greater employment of carbohydrate.

After work of 1056 kg.m. per minute, the respiratory quotient falls quite definitely below the preliminary resting value. In DOUGLAS's case this fall is more obvious in Experiment 9 than in Experiment 8, in which the work lasted for a much shorter period; it is also more marked than in Experiment 11 on HOBSON, notwithstanding the long period of work in this case. This is shown in Table XIII, which gives the preliminary resting respiratory exchange and the average respiratory exchange during the later period after the stop of the work when a fairly steady value is reached in the different experiments.

In Experiment 8 the oxygen consumption during the final steady period is on the whole slightly above, and the CO_2 production slightly below, corresponding values in the preliminary resting period. In Experiment 9 the alteration is in the same sense, but much more marked. In Experiment 11, however, both oxygen consumption and CO_2 production are lower than in the preliminary resting period, though the diminution of CO_2 production is proportionally greater than that of oxygen consumption. In Experiments 8 and 9 there was definite evidence of lactic acid formation and expulsion of preformed CO_2 during the work, and one might therefore expect that gradual retention of CO_2 after the work would account either in whole or part for the lowering of the respiratory quotient.

In our observations on the alveolar air changes, after this degree of work, we found (Table IX) that the aveolar CO_2 pressure reached the lowest point between the 4th and the 20th minutes after the stop of the work, and that recovery of the normal alveolar CO_2 pressure occurred within $\frac{3}{4}$ to 1 hour. As observations were only kept up for 43 minutes after the stop of the work in Experiment 8, retention of CO_2 might easily be sufficient to account for the lowering of the respiratory quotient. In Experiment 9, however, there is no definite indication of a gradual rise in the respiratory quotient, though observations were kept up for 79 minutes after the stop of the work, nor does Experiment 11 differ in this respect, though in this instance there were no variations in either the hyperpnœa or CO_2 output during the work, significant of lactic acid production. In BENEDICT and CATHCART's experiments a low respiratory quotient was also observed after exercise, and this was in some cases found to persist for as much as several hours.

It is therefore far from probable that the sole cause of the low respiratory quotient

after work in these last two experiments is CO_2 retention, and what appears to us more likely is that some at least of the diminution is due to the fact that when rest is resumed proportionally less carbohydrate than fat is metabolised than in the preliminary resting period owing to the depletion of carbohydrate during the work, a suggestion which is in consonance with the views expressed by the Zuntz school and by BENEDICT and CATHCART. It is, however, rather a remarkable feature that the fall of respiratory quotient after the stop of the work only becomes obvious in the experiments with work of 1056 kg.m. per minute, though Experiment 6 with work of 704 kg.m. per minute actually led to a greater total output of energy, owing to the longer duration of the work. In the experiments at 1056 kg.m. per minute, however, the work was done at a greater rate, and the average respiratory quotient was at a higher level during the work, so the actual effect on carbohydrate metabolism may have been greater. At first sight, one is liable to suggest that the high oxygen consumption in the final steady period of Experiment 9 may indicate some transformation of fat into carbohydrate to make up for the depleted stores of the latter, for this would entail some oxygen consumption without corresponding CO_2 production. Against this is the fact that in our experiments the energy output (calculated by the Zuntz-Schumburg Tables) is practically identical in the preliminary resting period and in the final stages of the rest following the work: *e.g.*, in Experiment 9 the calorie output per minute during the preliminary rest is 1·50 calories, and during the final steady period after work 1·55 calories, and in Experiment 8 these figures are 1·36 and 1·37 respectively.

In Experiment 12 with very severe work of 1232 kg.m. per minute the respiratory quotient falls to a very low value in the later stages of rest following the exertion, and for the last 57 minutes of the experiment is well below 0·7. The CO_2 output during the whole of this period is extremely low, especially if one considers it in relation to the oxygen consumption, and there seems little doubt that this is mainly determined by retention of CO_2 to make up for the large quantity of preformed CO_2 expelled at an earlier stage of the experiment. The average oxygen consumption during this period is rather higher than in the majority of the experiments, but this is mainly due to the high values in the last two determinations, and it is possible that these indicate a rise in the general metabolism owing to some accidental cause, *e.g.*, the subject may have got rather cold from sitting still so long (the period of rest after the work lasts in this case for almost an hour and a half). That CO_2 retention should continue for so long after the stop of the exertion is not in itself surprising, considering the severity of the work and the magnitude of the lactic acid effect, for, after exertion of similar severity (due to running up and down stairs repeatedly), CHRISTIANSEN, DOUGLAS, and HALDANE found (*loc. cit.*) that the alveolar CO_2 pressure was in one case still distinctly below the normal value 70 minutes after the work stopped, and the absorption power of the blood for CO_2 still depressed. The whole course of events in this experiment is very much like that observed by

DOUGLAS, HALDANE, HENDERSON, and SCHNEIDER (*loc. cit.*) in the Pikes' Peak expedition after ascending a quarter of a mile of a 1 : 4 gradient in 5 minutes at an altitude of 14,000 feet. This led to a great lowering of the respiratory quotient after the stop, the diminution being due to reduction of CO_2 output rather than to alteration in oxygen consumption. The respiratory quotient reached its minimum on this occasion half to three-quarters of an hour after the stop, and then rose slowly again, though a normal respiratory quotient was reached within an hour and a half in only one experiment.

It will be seen from Tables II–VI that in Experiments 4 and 6 the CO_2 percentage in the expired air and the depth of the breathing are about the same both in the preliminary resting period and in the last steady period after the stop, and that in Experiment 11 lowering of the expired CO_2 percentage in the later periods of the experiment is accompanied by a diminution in the depth of the breathing. In none of these cases does there seem to be any material alteration in either the dead space or the alveolar CO_2 percentage. In Experiment 9, and especially in Experiment 8, there is, however, a marked diminution in the expired CO_2 percentage in the final steady period after the stop below the value shown in the preliminary resting period, though the depth of the breathing remains much the same. This must indicate either an increase in the dead space or a lowering of the alveolar CO_2 pressure below their preliminary resting values. From the observations given in Table IX, one would not have expected a lowering of the alveolar CO_2 pressure to have lasted so long, or to have been of such magnitude, as to have made so great and lasting a difference in the expired CO_2 percentage, though it is true that the minimum percentage of CO_2 in the expired air is reached in Experiment 9 during the period when we might have expected the alveolar CO_2 pressure to be at its minimum. In Experiment 12 again the fall in the expired CO_2 percentage is more than can be accounted for by the small depth of the breathing, though in this case there is every reason to suppose that the alveolar CO_2 pressure was a good deal below the normal value. We feel, however, that further investigations are necessary on this point before a definite opinion can be formed.

A good deal more work is obviously necessary before we can get a clear picture of the course of events in these comparatively short periods of muscular work. The problem which appears to need most urgent solution is the part played by want of oxygen, for our present knowledge is still very inexact in this respect. It is evident that caution must be used in reckoning the energy output during short periods of hard muscular work, as well as during the earlier stages of rest following on the work, since the respiratory quotient is liable to show large temporary alterations which are dependent on other factors than an alteration in the proportion of carbohydrate to fat metabolised, such alterations involving great variations in CO_2 output without corresponding variations in oxygen consumption.

Conclusions.

(1) A simple method is described which enables a practically continuous record to be obtained of the respiratory exchange and hyperpnœa during and after muscular work on a stationary ergometer. It is possible by this means to get a considerable amount of information regarding rapid and transitory variations in the respiratory exchange.

(2) The efficiency of the body determined by this method agrees closely with the results obtained by other observers.

(3) Evidence is given in favour of the view that muscular work not infrequently involves the metabolism of a higher proportion of carbohydrate to fat than is the case during rest.

(4) With the harder degrees of work the course of events is liable to be influenced by the effects of serious shortage of oxygen as indicated by the production of lactic acid, which leads to a temporary great exaggeration of the hyperpnœa, accompanied by washing out of preformed CO_2 from the body and an abnormally high respiratory quotient. Our experimental data are not sufficient to exclude the possibility of the action of a slight degree of oxygen deficiency (possibly a "direct" action on the respiratory centre) in the lighter degrees of work in which there is no definite evidence of the production of lactic acid.

(5) After the stop of the work the respiratory exchange and hyperpnœa diminish with great rapidity. The respiratory quotient shows an immediate, but quite temporary, sharp rise, and it is suggested that this is largely due to the expulsion of CO_2 dammed back in the body during the muscular work owing to the alveolar CO_2 pressure being above the threshold stimulating value for the respiratory centre which prevails just after the cessation of the work, though this effect may be exaggerated by the simultaneous action of lactic acid. After this the respiratory quotient may return to the same value that it had previous to the muscular work, or it may show a marked diminution indicative of retention of CO_2 to make up for the preformed CO_2 washed out of the body at an earlier stage, or of a true change in the tissue metabolism dependent on the depletion of carbohydrate during the work.

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II.—*The Microscopical Features of Mechanical Strains in Timber and the Bearing of these on the Structure of the Cell-wall in Plants.*

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Communicated by Prof. W. H. LANG, *F.R.S.*

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[PLATES 1–4.]

INTRODUCTION.

When wood is subjected to increasing mechanical stress its component parts ultimately undergo changes resulting in permanent deformation or failure.* This paper deals with the minute microscopic changes upon which the permanent deformation of wood depends, and with the relationship between the microscopic structure of wood and some of its more important mechanical properties. The work has provided abundant material for a study of the structure of the walls of the cells of which wood is composed. Considerable light has been thrown on the structure and properties of the vegetable cell-wall in general, and it has been possible, from the facts for wood, to frame a hypothesis which seems to explain satisfactorily most of the properties of cell-walls.

The examination of the mode of failure in timber, which has led to the work described in this paper, was begun at the request of Lt.-Col. JENKIN, R.A.F. I am deeply indebted to him for the original suggestion of this line of work and for his continued interest during its course. I have derived great advantage throughout the work by discussing various engineering questions, as they have arisen, with Major ROBERTSON, R.A.F., and I desire to thank him for many helpful suggestions. I am further indebted to him for the supply of many fractured specimens of wood. The research has been carried out, in the course of more definitely applied work for the Air Ministry, in the Barker Cryptogamic Research Laboratory of the University of Manchester. I have to thank Prof. W. H. LANG, F.R.S., for encouraging me to attempt to relate the facts of the mechanical properties of wood to fundamental botanical questions bearing on the structure of cell-walls in plants.

The mechanical properties of a wood may depend on the microscopic or ultra-microscopic structure of the substance of the walls of the cells comprising it, on the form of these cellular elements, and also on their gross anatomical arrangement.

* It is recognised that a long thin column of wood may undergo mechanical failure by elastic instability, but since the deformation in such cases occurs within the limits of elasticity and is not permanent, the failure is accompanied by no permanent microscopical changes in the cell-walls of the wood. In this paper it is the changes accompanying failure by permanent deformation which are being considered.

In the present study it was found that the gross manner of failure in end-compression of some of the lighter coniferous woods differed in a striking way from that seen in many of the denser woods, both of Dicotyledons and of Conifers. It is probable that these gross differences are dependent on anatomical differences in the various woods, and are especially connected with the greater or smaller proportion of thick-walled elements, as well as with the form of the cells in the latter. On the other hand it is shown below that, underlying the gross differences in the manner of failure in end-compression in the various woods, there is a fundamental similarity in the method by which failure is initiated in the cell-walls of all woods. The earliest recognisable stages in the failure, due to the permanent deformation of the walls of the cells of the wood, are microscopic, and (requiring to some extent, special methods of demonstration) have not previously been described.

The macroscopic as well as some microscopic features of the failure of wood have been described by a number of investigators and brief reference must be made to the main results of their work.

THIL* (1900) described the anatomical structure of many woods in relation to their mechanical properties. He concluded that the form of the fracture depended on the medullary rays acting as places of less resistance and attempted to relate the course of the fracture to a spiral arrangement of the medullary rays.

JACCARD† (1910) investigated the anatomical structure of a large number of woods after compression. He studied the wood both of Dicotyledons and of Conifers and concluded that there is no specific type of rupture common to all woods and, contrary to THIL, that the fracture bears no relation to the medullary rays. The rupture is determined by points of least resistance in the wood, the pits in the walls of the tracheides and fibres forming such points of weakness. JACCARD described certain alterations in the folded cell-walls. He states that these show a longitudinal fibrillar structure which, in transverse sections, appears as a concentric layering in the walls. He attributes the appearances to the layers of the cell-walls having planes of less cohesion between them; under compression these planes behave as planes of cleavage and separation takes place along them. In the tracheides of the autumn wood of certain Conifers a fine transverse striation is referred to, but its significance is not discussed. Mention is also made of the similarity of some of the transverse folds he observed, to the dislocations described by VON HÖHNEL‡ for flax and other fibres.

FULTON§ (1912) studied the failure of Oak, Pitch Pine, Ash and Box, giving

* THIL, A., "Constitution anatomique du Bois."—'Étude sur les Fractures des Bois dans les Essais de Résistance.' 'Études présentées au Congrès Internat. des Méthodes d'Essai des Matériaux de Construction,' Paris, 1900. (Seen summarised in 'Le Bois,' Beauverie, 1905.)

† JACCARD, P., "Étude anatomique des Bois comprimés," 'Mitt. d. Schw. Centralanstalt für d. Forst. Versuchwesen,' vol. 10, heft 1, pp. 53–101, Zurich, 1910.

‡ HÖHNEL, VON, 'Jahrb. für Wiss. Bot.,' 1884, p. 311.

§ FULTON, A. R., "Experiments to show how Failure under Stress occurs in Timber, its Cause, etc.," 'Trans. Roy. Soc. Edin.,' vol. 48, Part II, p. 21 (1912).

illustrations of the external and of some microscopic characters of the failures he obtained. The manner of failure of specimens fractured in compression, in tension, and by bending was investigated, and FULTON concluded that "the initial cause of failure in all timber lies in the medullary rays." This failure is attributed to an initial set caused by the natural sinuous displacement of the fibres in a tangential plane round the medullary rays, and by the lack of cohesion between the fibres and the medullary rays. No details of any internal microscopic changes in the substance of the woody walls are given.

BRUSH* (1913) investigated the behaviour of the fibres of ten species of wood, in compression, tension, longitudinal shearing and bending. He found that for end-compression the fibres behave as hollow tubes and either buckle sharply or bend gradually. Whether a fibre buckles or bends depends on the thickness of its walls and on the moisture-content. Apart from this distinction between buckling and bending,† no description is given of more minute changes in the structure of the walls. No reference is made to the part played by the pits nor is the effect of the medullary rays considered.

RECORD‡ has summarised the results obtained by JACCARD and BRUSH but adds nothing to their descriptions.

MATERIAL, METHODS, ETC.

Preliminary observations indicated that the problems involved in the mechanical failure of wood could be best elucidated by a detailed study of a few woods of different types. For the purpose of this investigation, therefore, the woods of Silver Spruce (*Picea sitchensis*), Ash (*Fraxinus excelsior*), and Pitch Pine (*Pinus palustris*) were selected. For the elucidation of special points and for the verification of the main conclusions of this paper a number of other woods including Mahogany, Swamp Cypress, Oregon Pine, Larch, Birch and also Andaman Padouk have been examined.

The mechanical stress which has been mainly considered is longitudinal compression, *i.e.*, compression applied endwise and therefore acting in the direction of the grain of the wood. In addition, longitudinal tension and longitudinal shearing, both in radial and tangential planes, were considered for comparative purposes. Short rectangular test-specimens were used in the earlier stages of the research, but for most of the work on the effect of longitudinal compression, small cylindrical test-specimens with expanded flanges at either end were prepared. These specimens measured 1 inch long by $\frac{1}{4}$ inch diameter at the narrowest portion. The end-compression was applied in a small testing machine designed and constructed for the purpose by Major

* BRUSH, W. D., "A Microscopic Study of the Mechanical Failure of Wood," 'U.S. Depart. Agric. Review Forest Service,' vol. 2, pp. 33–38 (1913).

† In the present paper the terms "crinkling" and "buckling" are respectively equivalent to BRUSH'S "buckling" and "bending."

‡ RECORD, S. J., 'The Mechanical Properties of Wood,' New York, 1914.

ROBERTSON, R.A.F., to whom I am also indebted for the design of the test-specimen. The shape of the test-piece ensured that failure should occur in a middle region.

The shape of the specimens fractured in tension and in longitudinal shear, and also the method of test adopted in the latter case, are dealt with in the sections of the paper concerned with tension and with longitudinal shear.

True radial and tangential longitudinal sections were cut through the pieces after failure, and prepared for microscopical examination in the usual way. In order to retain the tissues and cells of the wood in the condition in which they were on removing from the testing machine and to prevent, as much as possible, expansion and readjustments in the tissues and cell-walls, the use of water was in most cases entirely avoided. The sections were cut from specimens soaked in absolute alcohol and the stains used were applied in solution in absolute alcohol. In using reagents like chlor.-zinc-iodide such treatment was not possible, but, by comparison with sections which were never wetted with water, it was possible to make allowance for the swelling and readjustment which took place.

GROSS FEATURES OF COMPRESSION FAILURE.

The gross appearances accompanying the compression failure in Spruce, Pitch Pine, and Ash will first be briefly described and then the detailed microscopic features of the failure will be dealt with for these woods.

In Spruce, after compression, a distinct zone of failure can be observed with the naked eye on both radial and tangential faces of a rectangular test-specimen. Plate 1, fig. 1, shows the appearance of the narrow zone of failure on the tangential face of such a compressed specimen. The zone is slightly inclined to the horizontal, its two edges are parallel, and there is no obvious displacement of the elements of the wood in the tangential direction as seen on this face. Plate 1, fig. 2, shows the zone of failure on the radial face of the same specimen. In this case the zone is practically horizontal, but its edges form a gently undulating outline. There is a most obvious displacement in the radial direction, this having been produced by the pushing over of the autumn wood of one year into the spring wood of the next. In some examples the displacement was so great that a separation of the tracheides took place at the annual rings (Plate 1, fig. 5*r*). The thin-walled tracheides of the spring wood in the zone of failure are crinkled and thrown into folds, whilst the thick-walled tracheides of the late wood are buckled more gradually. These differences, in the gross expression of the failure in the two kinds of tracheides, account for the undulating outline of the zone of failure which has been referred to above. In Spruce, there is no rupture between the medullary rays and the tracheides, nor indeed is any rupture visible on the tangential face of a compressed specimen. It will be seen later that Spruce differs in this particular from Pitch Pine, from Ash, and also from many of the woods, the failure of which has been described by the investigators referred to above.

In Pitch Pine, the zone of failure, as seen on a tangential face, is inclined at an angle of about 45° to the longitudinal axis of the specimen, the displacement of the elements of the wood having taken place in the tangential direction (Plate 1, fig. 3). The medullary rays are not of uniform size in Pitch Pine, and the separation takes place at the larger rays first (Plate 1, fig. 4). Owing to this separation at the medullary rays it is only possible to study the failure from tangential sections, since radial sections, except of specimens in very early stages of failure, separate completely into two parts. As in Spruce, the failure is manifested by buckling of the walls of the autumn wood and by crinkling of the walls of the spring wood. The proportion of thick-walled late wood, however, is much greater in Pitch Pine than in Spruce, and this probably accounts for the gross differences in the manner of failure.

In Ash, the gross features of the compression failure somewhat resemble those seen in Pitch Pine. The zone of failure is inclined at an angle, which may vary from 60° to 45°, to the longitudinal axis of the specimen. The failure manifests itself by the gradual buckling of the fibres, and this is accompanied by a displacement of the elements of the wood in the tangential direction (Plate 1, fig. 6). As in the failure of Pitch Pine, a separation of the elements ultimately occurs at the medullary rays. In Ash the fibres of the late wood comprise the main mechanical elements of the wood. When, therefore, a test specimen is compressed, the deformation of these fibres is responsible for the collapse of the specimen. As in Pitch Pine, the proportion of the thick-walled mechanical cells in the wood is very high when contrasted with a wood like Spruce.

These observations, on the grosser features of the failure of Pitch Pine and of Ash, confirm the descriptions given by FULTON for these two woods, but, as will be seen below, the present paper is not in agreement with his interpretation of the cause of failure. In Spruce, even the gross features of the failure are not in agreement with FULTON'S explanation, since the displacement is in the radial direction and separation never occurs at the medullary rays. It has been found that the actual causes of failure must be traced back to the initial stages of deformation which become manifest in the cell-walls of the tracheides, or fibres of the wood. This is the case, even though the final, grosser characteristics of the failure in woods like Pitch Pine and Ash, may partly be determined by the size and distribution of the medullary rays. From the results described below, it is likely that in all woods these initial stages of the failure and their relations to the minute structure of the woody walls are of primary importance.

That the initial stages of the permanent deformation have either been entirely overlooked, or that their significance has not previously been realised, may account for the diversity of the explanations of the manner of failure offered by different investigators. The grosser features of the failure of all three woods studied in detail, and of a large number of others examined more briefly, are secondary effects dependent on the anatomical structure of the particular wood. The initial failure, in all

cases, depends on primary changes, in the substance of the cell-walls, which will be described below.

Staining Reactions in the Zone of Failure.

Before describing the more minute changes associated with the permanent deformation, reference must be made to some unexpected results which were first obtained during the preliminary study of the grosser features of the zone of failure in compressed Spruce. Similar results were later obtained for Pitch Pine, for Ash, and for a large number of other woods. It was found that the zone of failure behaved differently, towards various stains and reagents, from the uninjured parts of the wood. Plate 1, figs. 7 and 8, taken from sections through specimens of compressed Spruce, illustrate this differential behaviour of the zone of failure when the reagent used is chlor.-zinc-iodide. The zone of failure appears as a deep blue band in contrast to the yellow stain given by the unaltered wood. The blue stain is similar to that usually obtained when chlor.-zinc-iodide acts on pure cellulose. Observation, by high powers of the microscope, shows that the blueness of the zone is due to transversely running bars as seen on the surface walls of the tracheides (Plate 2, figs. 15 and 16). These blue bars are separated by narrow yellowish areas; within the actual zone of failure they are very numerous and close together, but they also are present in a more diffused manner for a considerable distance beyond the zone of failure (Plate 2, figs. 15 and 16). The blue reaction extends into the depth of the walls, and in the section of these the change is manifested by obliquely running bars of blue (Plate 2, fig. 15*x*).

An attempt was made, by using a variety of histological stains and reagents, to elucidate the nature of the change in the fractured region. Iodine and sulphuric acid give results parallel with, but even more striking, than those with chlor.-zinc-iodide. As before, the altered zone stands out in contrast to the normal parts of the wood. The deformed region gives a dark green colour rather than the blue which would have been expected if the zone consisted of pure cellulose. The rest of the wood, consisting of unaltered tracheides, gives the normal deep yellow colour. A close examination of the region of failure shows that the yellow lignin-reaction is present in the zone of failure, but that this yellow colour is largely overpowered by a blue colour in the portions which stand out blue with chlor.-zinc-iodide. The dark green stain is the result of the combination of the blue with the yellow of unchanged lignin.

Parallel results were obtained with a number of stains and reagents, and the effects of these are summarised in the following Table:—

Stain or Reagent.	Zone of failure.	Unaltered wood.	Remarks.
Chlor.-zinc-iodide	Blue	Yellow	
Iodine and H_2SO_4	Dark green	Yellow	
Aniline chloride	Yellow	Yellow	Normal lignin-reaction throughout.
Phloroglucinol and HCl	Pink	Pink	Normal lignin-reaction throughout.
Aniline chloride followed by aniline blue	Blue or bluish green	Yellow	
Cotton red (carthemin) followed by aniline blue	Blue	Red	
Safranin followed by hæmatoxylin	Blue	Red	Contrast less sharp than in other cases.

The results obtained, using aniline chloride alone, and also those with phloroglucinol, especially point to the presence of substances giving the lignin-reaction in the zone of failure, possibly in undiminished quantity. On the other hand, the behaviour of the walls of the fractured tracheides, especially towards chlor.-zinc-iodide, iodine and sulphuric acid, and such aniline stains as aniline blue, suggests that in some way the cellulose present in the lignified walls has been unmasked as a result of the mechanical strain. This change in the staining properties of the walls of compressed tracheides has obviously a fundamental bearing on the nature of the process of lignification of the cell-wall, and it will be further discussed in this connection after the minute details of the structure of the deformed walls have been described.

Apart from slight individual differences in the staining properties of the walls of the tracheides, fibres, and vessels of different woods, it was found that, broadly speaking, the changes in the behaviour towards reagents and stains just described for Spruce held for the zone of failure in such widely different woods as Pitch Pine, Ash, Birch, Andaman Padouk, Oregon Pine, and Swamp Cypress. Whenever failure in compression occurs in wood, it is possible to demonstrate the region of failure, however slight this may be, by making use of stains and reagents. The method has been particularly useful in demonstrating the very earliest stages in the deformation of the walls of the tracheides and fibres. It has thus been possible to trace the sequence of changes in compressed specimens from the initiation of deformation up to the stage when a clear zone of failure is visible on the faces of the specimen. This sequence of changes will first be described for Spruce.

Initial Stages of Failure in Spruce.

Test specimens were carefully compressed so that only the first signs of failure (*i.e.*, that the elastic limit had been reached and that yielding was taking place) were

evident from the behaviour of the testing machine. Sections from such specimens gave early stages in the development of the zone of failure. Plate 2, fig. 10, is the tangential view of such early stages as they appear in the relatively thin-walled spring wood. The walls of the tracheides are locally crinkled, and the crinkling extends in an approximately horizontal direction across the specimen from tracheide to tracheide. In the autumn wood, the walls of the tracheides buckle more gradually than the thinner walls of the spring wood, and are not thrown into abrupt folds like the latter.

Corresponding radial sections show the walls of the tracheides of the spring wood crinkled as in the tangential sections, and, as before, the series of local crinklings forms a horizontal band across the spring wood (Plate 2, fig. 11). The thicker walls of the tracheides of the late wood buckle gradually, and the line of the failure is usually inclined at about 45° to the length of the tracheides (Plate 1, fig. 6). Both in the tracheides of the autumn wood and in those of the spring wood, the pairs of walls of adjoining tracheides appear to behave as one, since they invariably buckle or crinkle, as the case may be, in the same direction. The ratios of the thickness of the cell-walls to the diameter of the cavities probably determines whether the crinkling seen in the spring wood, or the simple buckling seen in the autumn wood, will occur. In the passage from the spring wood to the autumn wood of the same year, the change in the thickness of the walls is a gradual one, and, in consequence, the transition from crinkling to buckling is also gradual.

The medullary rays in Spruce crinkle or buckle with the tracheides, but do not appear to form special places of weakness in the wood (Plate 2, fig. 10, *m.r.*). No separation between the tracheides and the medullary rays has ever been observed in Spruce.

The first lines of failure may pass through bordered pits as the former extend across the specimen, but no evidence was obtained that the pits form special places of weakness in the wood. Many very early stages in the failure were obtained, however, which prove conclusively that the yielding in the substance of the walls takes place, apart altogether from the presence or absence of pits. In Spruce, if the bordered pits formed special places of weakness in the woody walls, we should expect the radial walls, on which the bordered pits mostly occur, to be weaker than the tangential walls. The displacement of the elements of the wood should, on this supposition, take place in the tangential direction. It has, however, been shown above that this is not the case in Spruce, and it will be shown below that the fundamental changes that accompany the deformation of the cell-walls are not connected with the presence or with the distribution of pits. In this respect the present work is not in agreement with the conclusions of JACCARD, or with the statements of RECORD regarding the weakening effect of the pits.

Minuter Changes preceding Buckling and Crinkling.

The buckling and the crinkling of the walls of the tracheides of the autumn and spring wood respectively are preceded by local changes in the substance of the cell-walls. These changes can readily be observed in the radial and tangential sections of Spruce wood, showing initial failures. Somewhat thick radial longitudinal sections of Spruce show the thickness of the tangential walls of the tracheides in section and the radial walls in surface view. The changes which lead to deformation consist in the appearance of extremely fine, but sharply defined, crack-like lines in the walls of the tracheides.* In the sectional views both of the radial and tangential walls of autumn and spring wood, the lines are somewhat irregularly cross-hatched, and run obliquely through the secondary layers of the walls at inclinations greater than 45° to the edge of the wall (Plate 3, figs. 1, 2, and 4; Plate 2, figs. 17 and 18). It is clear, that where each line comes out to the surface, there is a step-like projection of the wall-substance (Plate 3, figs. 1 and 2). In most cases, the direction of the lines is common to the secondary layers of pairs of walls of adjoining tracheides, but no line has been observed crossing the middle lamella between adjoining tracheides. This probably signifies a difference in the elastic properties of the middle lamella (Plate 2, fig. 17, *ml.*).

In the surface view of the walls of the spring wood, corresponding to the sectional views just described, the lines run across the walls in an approximately horizontal or transverse direction (Plate 3, figs. 1 and 4). On the surface of the walls of the autumn wood they may be either transverse, or more or less inclined to the axis of the cell, often forming a system of fine cross hatchings (Plate 3, fig. 6). The inclination of the lines, as seen on the surface of the walls, is never the same as that of the slits of the pits on the walls of the tracheides. The appearances of the lines, in the sectional views of the cell-walls and also in the surface view of these, are consistent with an explanation which regards them as the traces of planes of displacement in the cell-wall substance. The boundaries between the step-like projections, seen in the sectional views, manifest themselves as one or more series of parallel, transverse or inclined lines on the surface of the walls (Plate 3, figs. 6 and 7).

When deformation begins in the cell-walls of the wood, parts of the substance of the walls are pushed over other parts so that regular planes of slipping are locally developed. That the lines which represent the boundaries between these planes of

* It has been found that the mere mechanical effect of cutting sections, however good the knife may be, is sufficient to produce artificially a small number of these lines of slip, but tracing the appearances back from advanced to more initial failures leaves no doubt that the lines described are a direct result of the compression. In a number of instances sections were alternately cut from control uninjured specimens of wood and from the compressed specimens. The comparison of these afforded additional evidence of correctness of the interpretations given. Tracheides, isolated by maceration from a zone of failure, with practically no mechanical manipulation, show slip-lines in very large numbers in the buckled or crinkled walls, and only a few elsewhere.

slipping are sometimes inclined approximately at 45° to the axis of the cell, indicates that they are probably planes of shearing in the substance of the walls.

As the failure proceeds, the number of shearing or slipping planes rapidly increases, with the result that an extended region of the wall is affected, until finally the wall buckles or is thrown into a series of crinkles (Plate 3, figs. 3, 4, and 5). The walls, under the stress of end-compression, thus exhibit plasticity by the development of planes of slip or shear within their substance. It is by this microscopic, plastic deformation of the substance of the cell-walls that the failure is initiated. Whether buckling or crinkling subsequently occurs is determined by the relative thickness of the walls of the tracheides (Plate 3, figs. 3 and 5).

Very large numbers of early stages were observed in which slip-planes had developed before any visible buckling or crinkling had begun to take place (Plate 2, fig. 17 ; Plate 3, fig. 1). There is, therefore, no doubt that the primary mode of failure, or permanent deformation, is by the development of the microscopic planes of shear in the substance of the cell-walls. The buckling and the crinkling are subsequent developments.

After buckling and crinkling have taken place, the further development of the failure is then determined by the anatomical structure of the wood. In Spruce, as has already been pointed out, the relatively small proportion of autumn wood is probably one of the main factors in determining the ultimate and grosser characters of the failure.

The "slip-lines" or planes described here for the wood of Spruce present certain similarities to the slip-bands or gliding planes described by EWING and ROSENHAIN* for the crystals of metals under strain, and by REUSCH† and others at an earlier period for many crystalline substances. Since wood cannot be regarded as crystalline in the ordinary sense, the relationship between the phenomena in wood, on the one hand, and in the metals and crystals on the other, will be discussed below.

By careful illumination it is possible to observe the slip-lines described above, in unstained sections merely mounted in water. A more vivid demonstration of them, however, is obtained by staining compressed specimens with aniline chloride followed by aniline blue. These stains afford a means of demonstrating the lines even in the most initial stages of failure, before any buckling or crinkling has taken place, and before a zone of failure has developed. The lines and the wall-substance in their immediate vicinity hold the blue stain, whilst the rest of the wall remains yellow. Corresponding results are obtained with cotton red and aniline blue, and with chlor.-zinc-iodide solution. It has in fact been established that the remarkable differential staining of the zone of failure described above (pp. 54 and 55) is almost entirely due to the multiplicity of slip-planes in the substance of the cell-walls. The substance of

* EWING and ROSENHAIN, 'Phil. Trans.,' A, vol. 193 (1900); ROSENHAIN, 'Introduction to Physical Metallurgy,' 1914.

† REUSCH, quoted in LEHMAN'S 'Molekularphysik,' vol. 1, p. 64 *et seq.* (1888).

the walls in the immediate vicinity of these microscopic slipping planes holds the cellulose stains and reagents selectively.

The staining effect is thus clearly connected with the displacement of the particles of the cell-walls in the vicinity of the slip-planes and strongly suggests the possibility of a local chemical alteration of the lignified wall at these places. On the other hand, the modification in the staining properties may merely be the result of a physical alteration in the wall-substance. It is possible that the alteration is due to an actual transformation of the lignified wall into cellulose, in the immediate vicinity of the planes of slipping. It must be pointed out that many of the staining effects obtained with ordinary dyes on cell-walls, as well as the usual reactions for cellulose, can be explained as adsorption effects. BARGER* and his collaborators, for example, have shown that iodine forms blue adsorption compounds, with a large variety of organic chemical compounds in addition to that formed by the action on starch. He has also shown that the ability of an organic substance to form such blue adsorption compounds depends on the particles of the substance being in the colloidal state. The blue colour produced in the chlor.-zinc-iodide reaction for cellulose is probably due to the formation of such an adsorption compound with iodine in the presence of zinc chloride. Lignified cell-walls normally give no such blue compound with iodine in the presence of zinc chloride.

Botanists, since the time of NÄGELI, have regarded the lignification of the cell-wall as due to the passage of incrusting substances into the substance of the cellulose wall. Expressing this view of the nature of lignification in terms of the more modern conceptions of colloid chemistry and physics, the incrusting process would consist in the adsorption of lignone substances by the colloidal particles of cellulose in the wall. The formation of such adsorption compounds would lead to the complete obscuring of each particle of cellulose by a film of lignone substances Such a compound would, therefore, be incapable of forming blue adsorption compounds with iodine.

It is possible to think of such adsorption compounds of cellulose with lignone substances in the cell-walls of wood, as being split up by mechanical stress and the cellulose being revealed as a consequence. Such a view would explain why, even in the region of failure, the ordinary lignin-reactions can be obtained as readily as in the normal wood, but side by side with a strong cellulose-reaction. According to this explanation the mechanical strain does not result in the destruction of the incrusting substances, but merely in their ultramicroscopic rearrangement in relation to the cellulose particles by which they can be regarded as being adsorbed in the unaltered lignified walls.

* BARGER, C., and FIELD, 'Journ. Chem. Soc. Trans.,' 1912, p. 1394; BARGER, G., and STARLING, W., 'Journ. Chem. Soc. Proc.,' 1913, p. 128.

Behaviour of Compressed Wood Towards Polarised Light.

The facts regarding the nature of the deformations in the cell-walls of compressed specimens and the interpretations which have been outlined above, received striking confirmation by the use of the polariscope. It was found that the optical properties of the walls of the deformed tracheides had been altered in important respects.

Under low powers of the microscope, with crossed nicols, the zone of failure, both in radial and tangential sections, stands out as a very bright band across the unaltered parts of the section. The band corresponds exactly in form to similar bands brought out by staining methods in other specimens. On rotating the analyser to the parallel position, the illumination of the band reverses and stands out as a dark zone crossing a brightly illuminated section. Comparison with the behaviour of the uninjured parts of the wood shows that in addition to the illumination being reversed in the zone of failure, the degree of illumination is brighter than in the unaltered parts of the wood at their brightest.

The effect under polarised light is partly due to the folding of the cell-walls and consequent altered orientation of these, but examination of walls showing slip-lines without buckling or crinkling, shows that the folding is not the main cause of the altered anisotropy in the zone of failure. Under high magnifications the coarser slip lines are particularly well demonstrated and the parts of the wall substance in the immediate vicinity of the lines show greater anisotropy than the rest of the wall. This results in the lines standing out under crossed nicols as bright lines (often red) across the darker wall, and as dark lines in the parallel position.

The polariscope thus affords an even more effective means of demonstrating the slip-lines than when staining methods are used. Both methods give clear results for Spruce, to which the above descriptions apply, but, in some other woods like Ash the shearing lines are not so easily demonstrated by the methods of differential staining, owing to the unaltered walls taking up, to some extent, the cellulose stains and reagents. In such cases the polariscope has been particularly useful as a rapid and vivid means of demonstrating the existence of lines of slip.

Slip-Planes in Ash.

In Ash the gross characteristics of the failure, briefly referred to in an earlier paragraph, are preceded by changes in the walls of the fibres which comprise the main mechanical elements of the wood. It has been pointed out that the fibres buckle gradually under compression and finally there is a separation of the tissues at the medullary rays. The buckling of the fibres is, however, invariably preceded by the initiation of microscopic planes of slipping in the substance of the cell-walls.

The multiplication of these slip-planes leads to the buckling of the fibres as in the autumn tracheides of Spruce (Plate 3, fig. 9). The slip-lines, in the sectional views of the walls, are often inclined at an angle of about 45° to the long axis of the fibre,

but the angle, as in Spruce, may be considerably higher than this. Seen in the surface view of the walls the lines may cross transversely but are often inclined at a high angle to the longitudinal axis of the fibre; this inclination, however, is never that of the slits of the pits.

The first sign of failure, as in Spruce, is the development of these planes of shear in the cell-walls; as they increase in number and more fibres become involved the failure gradually passes over into buckling of the fibres to be followed by separation at the medullary rays. The whole process in Ash, however, is much more gradual than in Spruce, and a much greater area of fibres shows slip-lines before the buckling begins. This may be correlated with the greater plasticity of the wood of Ash than that of Spruce. Even in the autumn wood of Spruce the buckling is much sharper than is usual in Ash (Plate 1, *cf.* figs. 5 and 6).

Slip-Planes in Pitch Pine.

In Pitch Pine the thin-walled cells of the spring wood are crinkled under compression, while the thick-walled tracheides of the late wood buckle like those of the autumn wood of Spruce. The crinkling or buckling of the tracheides in this wood is also preceded by the development of definite shearing planes in the substance of the cell-walls. These are particularly well demonstrated both by the method of differential staining and by the polariscope. The sequence of changes, leading to buckling or crinkling, is similar to that described for Spruce and for Ash (Plate 3, fig. 8). The much greater proportion of thick-walled tracheides probably largely determines the grosser characters of the failure already described.

For the three woods studied in detail, it is thus clear that the first stage in the failure under compression is the development of planes of shearing in the substance of the walls of the cells comprising the wood. The buckling or crinkling are secondary effects, depending probably on the thickness of the cell-walls. The still grosser appearances of the failures can be explained by such anatomical characters as the proportion of autumn to spring wood, and the size, nature, and distribution of the medullary rays. The main contribution of this paper, however, is the tracing of the original causes of failure back to the minute microscopic deformation of the substance of the cell-walls of wood, along planes more or less definitely orientated in relation to the direction of the stress. The general conclusions regarding the origin of failure in compression derived from the detailed study of Spruce, Pitch Pine, and Ash, have been extended to all the woods so far examined. These include Oregon Pine, Oak, Swamp Cypress, Birch, Larch, and Andaman Padouk.

The Effect on Compression Failures of Soaking Timber in Water.

In the work described above, the moisture-content of the test specimens was that of ordinary air-dried wood, *i.e.*, from 12 to 15 per cent. of the dry weight of the

wood. It was found in a previous investigation that the compressive strength of Spruce is strikingly affected by the moisture-content of the specimen tested. For example, wood, which in the fibre-saturated condition (*i.e.*, about 30 per cent. moisture) yielded under a compressive stress of about 3000 lbs. per square inch, required about 8500 lbs. per square inch to produce failure when the moisture-content was reduced to 3 per cent. of the dry weight of the wood. It was of interest, therefore, to ascertain if this remarkable difference in the strength values at different conditions of moisture-content could be correlated in any way with differences in the microscopic characters of the strains. Some results from this point of view were obtained with specimens of Spruce soaked in water, to ensure complete fibre-saturation.

The soaked specimens were compressed in the testing machine until the indicator showed yielding had begun to take place. Sections from such specimens demonstrated that failure is initiated, as in air-dry wood, by the development of planes of slipping in the cell-walls. These slip-planes, however, are more diffused through the specimen than when dry wood is compressed. The zone of failure, which ultimately appears, is in consequence broader, and its limits are less sharply defined than before. All the appearances obtained in such soaked specimens suggest that the buckling and even the crinkling of the tracheides occur much more gradually since they are preceded by much more generalised slipping than when the Spruce is dry.

In Ash, the effect of soaking in water is even more striking than in Spruce. As before, the failure is initiated by the development of planes of slipping in the fibre walls. The initial failures so produced, however, occur in large numbers, evenly distributed through the length of the test-specimen (text-fig. 2). In Ash of ordinary moisture-content, *e.g.*, 15 per cent., the few initial failures which arise are confined to a middle region of the specimen, and lead to a zone of failure and displacement in this region (text-fig. 1). The form of the individual initial failures both in dry and in soaked Ash is the same, even though the number and distribution of these initial failures is so different.

The effect of soaking the wood of Ash and of Spruce in water is to lower considerably the value of the compressive strength of the wood, and to make it more plastic. This increased plasticity is manifested by the more ready occurrence of slipping in the substance of the cell-walls, and also by the more generalised distribution of the slip-lines in the test-specimen after failure.

In the employment of wood for certain purposes, the pieces are often shaped by bending, after soaking in warm water or steaming. The pieces are held in the bent position, and, on cooling and drying, retain the deformation given to them. This method is frequently employed in the case of Ash. It was therefore of interest to examine the microscopical effect of producing such permanent deformation by bending wood after soaking in warm water or steaming it.

Specimens of Spruce and of Ash, measuring 10 inches by ¼ inch by ¼ inch, were bent to various degrees, and held in the bent position until cold and dry. Sections through the middle of such bowed specimens showed that the permanent deformation had invariably been produced by the development of planes of slipping in the walls of the tracheides or fibres on the compression side of the specimen. These planes of slipping were regularly distributed through the deformed portion, being most abundant at the region of greatest deformation. In cases where extreme bending of the specimen was carried out, initial failures and buckling of the fibres and tracheides took place. Even where only the very slightest permanent bowing was produced, it was possible to demonstrate planes of slipping in the cell-walls in the middle part of the specimen. The facts for Ash and for Spruce, permanently deformed after soaking in warm water or steaming, are thus in entire agreement with those obtained in the compression failures of these woods in the air-dried condition.

Fractures in Longitudinal Tension.

The gross features of the tension fractures of a number of woods, including Oak and Ash, have been described and figured by Fulton (*loc. cit.*). He found that the slipping,* which resulted in fracture, invariably occurred along the planes of the medullary rays, so that the fracture had an irregular splintery character, as seen on the tangential face, and was manifested on the radial face, as a straight break across the specimen. The results obtained for the tension fractures of Spruce, Ash, and Pitch Pine, in the present work, are not in agreement with those of Fulton, even for the gross form of the fractures. As in the failures in compression, the initiation of the fracture has been traced back to the behaviour of the substance of the walls of the individual tracheides or fibres of the wood.

The fractured specimens, which were supplied me by Major Robertson, R.A.F., were cylindrical in form with expanded ends, the central portion of the specimen being much narrower in diameter than the ends which had been held in the grips of the machine.

The gross features of the fractures may be briefly summarised. In Spruce, the fracture is normally of a splintery character, as seen both from the radial and the tangential faces, but the planes of slipping for the main splinters are generally parallel to the annual rings, and the gross slip often takes place in the spring wood in the near vicinity of these rings (text-fig. 3). A certain amount of slipping also occurs in planes at right angles to the annual rings, and this explains the splintery character, as seen in the tangential view of the fractured specimen (text-fig. 3). The history of the development of the broad picture of the fracture is probably as follows. The initial effect of the tension stress is to cause local isolated ruptures in the specimen; these ruptures result in the production of longitudinal shearing

* The slipping referred to by Fulton is a gross slipping of the tissues of the wood and not the more microscopic slipping in the substance of the walls, described in this paper.

stresses, and the rest of the material fails by longitudinal shearing. The microscopic characters of the fractured surfaces, *e.g.*, at S (text-fig. 3), are identical with those obtained in specimens of Spruce fractured by longitudinal shearing, and are therefore consistent with the explanation given.* The microscopic characters of the initial local ruptures will be described below.

In Ash the fracture is more abrupt than in Spruce, but the gross slipping in this wood also takes place along planes parallel to the annual rings, particularly in the spring wood (text-fig. 4). As in Spruce, there is also some slip in the tangential planes, but this is not so obvious in the specimens I have examined as that in the planes parallel to the annual rings. Plate 4, fig. 15, shows that the form of the fracture is not affected by the medullary rays.

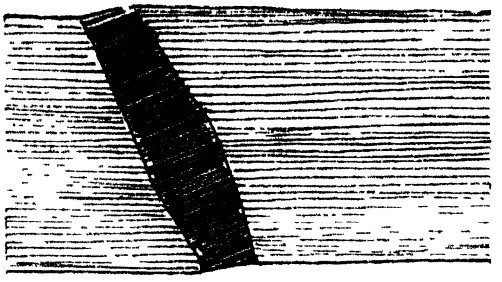
In Pitch Pine the tension fracture is usually extremely short and is transverse in character, but even here the bigger irregularities follow the planes of the annual rings rather than those of the medullary rays (text-fig. 5). Owing, however, to the normally brittle character of Pitch Pine, it is not so suitable for the consideration of the questions under discussion.

The more microscopic characters of the fractures produced by longitudinal tension may now be described. In tangential sections of the fractures of Spruce, Ash, and Pitch Pine, there is no evidence that the medullary rays form special places of weakness for pure tension stresses in the longitudinal direction. The medullary rays are so abundant that it seems unlikely that fractures could occur without involving some of them; but the specimens examined conclusively showed that the initiation of slipping could not in any way be specially related to the rays (Plate 4, fig. 18).

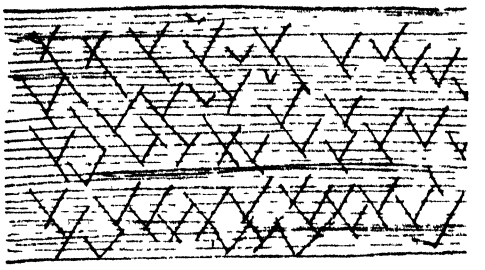
Normal specimens of Spruce in the air-dried condition give breaks of the tracheides similar to those shown in Plate 3, figs. 10 and 11. The walls of the tracheides, both of the autumn and spring wood, are ruptured along planes which have exactly the inclination of the slits of the pits. Plate 3, fig. 11, shows a complete fracture in a spring tracheide while at (S.) slipping, which has not yet led to actual rupture, is taking place. The planes of slipping in the walls and also the ruptures in these are confined to the vicinity of the pits, although the inclination of the slip-planes is the same as that of the slits of the latter. In tension failures, unlike compression failures, the initial rupture rapidly follows upon the development of planes of slipping in the substance of the walls; rupture is, therefore, preceded by the appearance of relatively few slip-lines. It is noteworthy, however, that the fractured ends of the tracheides, as well as the few slip-lines that appear, behave selectively towards stains and reagents just as do the slip-lines in the compression failures.

The slip-lines in the sectional view are generally obliquely inclined to the edge of the walls. From the general microscopic appearance of the fractured tracheides, however, there is no doubt that most of the slipping takes place in tangential planes (*i.e.*, at right angles to the surface) in the walls. Text-fig. 6 illustrates the direction

* See pp. 67–69 below.



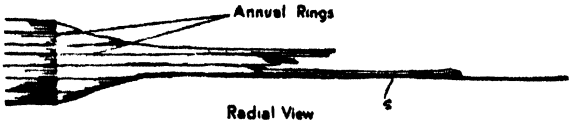
TEXT-FIG. 1.

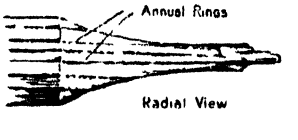


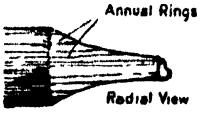
TEXT-FIG. 2.

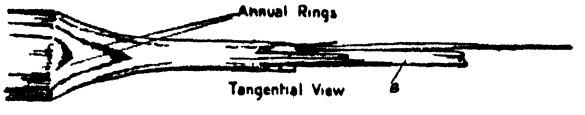
TEXT-FIG. 1.—Diagram of zone of failure in air-dry Ash (15 per cent. moisture). × 4.

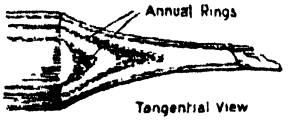
TEXT-FIG. 2.—Diagram of lines of failure in similar piece of Ash to that in fig. 1, after soaking in water. × 4.

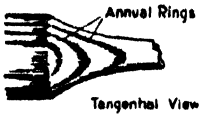










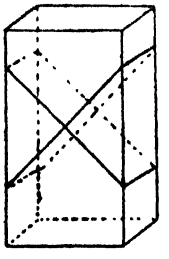


TEXT-FIG. 3. TEXT-FIG. 4. TEXT-FIG. 5.

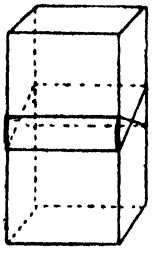
TEXT-FIG. 3.—Specimen of Spruce fractured in tension, showing appearance of fracture from the radial and tangential views. (Half actual size.)

TEXT-FIG. 4.—Similar specimen of Ash to that in text-fig. 1, fractured in tension. (Half actual size.)

TEXT-FIG. 5.—Similar specimen of Pitch Pine, fractured in tension. (Half actual size.)



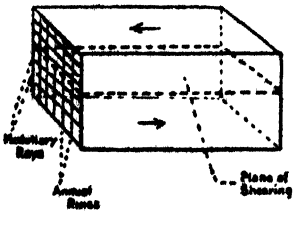
TEXT-FIG. 6.



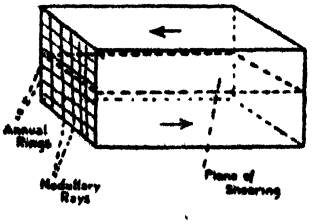
TEXT-FIG. 7.

TEXT-FIG. 6.—Diagram illustrating planes of shear in the cell-walls in tension specimens of Spruce.

TEXT-FIG. 7.—Diagram illustrating the direction of the planes of shear in the cell-walls of compression specimens of Spruce.



TEXT-FIG. 8.



TEXT-FIG. 9.

TEXT-FIG. 8.—Diagram to illustrate the direction of longitudinal shearing along a radial plane.

TEXT-FIG. 9.—Diagram to illustrate direction of longitudinal shearing along a tangential plane.

of the planes of slipping in such cases. This differs from the slipping in the radial direction in the walls (*i.e.*, parallel to the surface), which is most frequently observed in the compression failures of Spruce (text-fig. 7). In tension fractures the displacement of the wall-substance is thus in the tangential direction in the individual walls, whilst in compression failures the movement is usually in the radial direction.

For normal Spruce the substance of the cell-walls behaves differently towards tension and compressive stresses. In both cases the failure occurs by shearing in the wall-substance, but this is much less plastic in tension than in compression, since, in the former case, very few planes of slipping arise before rupture occurs. While in tension the special planes of weakness generally have the same inclination as the slits of the pits, in compression, slipping never occurs along these planes, but along planes orientated at right angles to them (*cf.* Plate 3, figs. 10 and 6).

Striking deviations from the form of tension fracture just described, were obtained for brittle samples of Spruce and also for the normal tension fracture of a number of the harder woods. In these cases the lines of fracture do not follow the inclination of the slits of the pits, but, on the contrary, the walls are broken across transversely, *i.e.*, at right angles or at a high angle to the long axis of the tracheide or fibre (Plate 4, figs. 13, 14, and 17). In the portion of the wall seen in section, it is clear that the break occurs along radial planes in the individual cell-wall, inclined at 45° or rather more to the long axis of the cell. This tension fracture of brittle wood thus occurs along planes of slipping, similarly orientated to those already described for compression specimens. Some early stages in such tension failures of a brittle sample of Spruce were obtained, and these indicated that even in this case the actual rupture was preceded by the formation of a very small number of planes of slipping in the walls of the tracheides. These planes of slipping rapidly connected up across the specimen, which then fractured (Plate 4, figs. 13 and 14).

The results obtained from tension fractures of brittle Spruce appear to indicate that the brittle qualities are associated with a greater resistance to slip along tangential planes, having the same inclination as the slits of the pits, than along radial planes in the individual walls. In normal Spruce, on the other hand, there is more resistance to slipping on radial planes than on tangential planes in the individual walls; this leads to the more splintery appearance of the fractured ends of the cells (*cf.* Plate 3, fig. 10, and Plate 4, fig. 14).

In Ash, the fibres are roughly circular in transverse section, and not, as in the tracheides of Spruce, rectangular in section. The slipping which takes place in the walls as a result of tension, occurs along oblique planes which seldom have the same inclination as the slits of the pits (Plate 4, fig. 15). As in Spruce, the tension failure occurs by slipping in the substance of the walls of the fibres, and rupture takes place before very many planes of slipping have developed. Some slight indications were obtained that more slip-planes appear in Ash before rupture than in Spruce, but it would not at present be safe to draw conclusions from this.

In Pitch Pine, the fractured tracheides present very close similarities to those seen in the brittle specimens of Spruce. The abrupt break shown in text-fig. 5 is accompanied by transverse breaks as seen on the surface of the walls of the tracheides (Plate 4, fig. 16). In this case, also, the sectional view of the walls shows slip-lines inclined at about 45° to the edge of the wall. The failure, therefore, occurs by slipping along radial planes in the substance of the cell-walls, but extremely few slip-planes are developed in any one tracheide, prior to rupture taking place (Plate 4, fig. 16).

The facts for the tension failures of Spruce, Ash, and Pitch Pine are in harmony with those already described for the compression failures of these woods. Failure takes place, both in tension and compression, by the development of planes of slipping or shearing in the substance of the cell-walls. In tension failures of brittle samples of Spruce, of normal Pitch Pine and generally of normal Ash, the planes of slip are identical with those obtained in compression. In normal Spruce and occasionally in Ash, the planes of shearing for tension failures have the same inclination as the slits of the pits. Most of the examples of failure in tension studied have shown only a very localised distribution of slip-lines in the vicinity of the fracture.

Longitudinal Shearing.

The behaviour of wood subjected to longitudinal shearing can be correlated with the behaviour in longitudinal tension. Specimens of Spruce were fractured by longitudinal shearing both in the tangential and in the radial directions. Small rectangular pieces measuring about $\frac{1}{2}$ inch long by $\frac{1}{4}$ inch by $\frac{1}{4}$ inch were broken by pushing one part of the specimen over the other by striking a blow on the specimen held firmly in a vice. The fractures obtained by this simple method corresponded exactly in their gross and microscopic characters to those exhibited by larger specimens tested in longitudinal shearing by Major Robertson, R.A.F. It was found advantageous to use this simple method of applying the stress, since it was then possible to make microscopical observations on a large number of specimens. It was also possible to trace earlier stages in the failure than could possibly have been obtained in specimens from a testing machine.

Text-figs. 8 and 9 illustrate the directions in which the specimens were cut, and the arrows indicate the direction of the shearing. When Spruce is subjected to shearing along a tangential plane the failure always occurs in the spring wood and at rupture the fractured surfaces are finely hairy. Microscopical examination shows that the fracture occurs in the substance of the cell-walls of the tracheides and not by a separation of the tracheides from one another. Radial longitudinal sections through the fracture show that the substance of the cell-walls has been torn into a series of oblique shreds (Plate 2, fig. 12); these shreds give rise to the hairy appearance mentioned above.

The shearing stress in the tangential plane has resulted in the separation of the substance of the walls along planes which are usually inclined at an acute angle to the

long axis of the cells (Plate 2, fig. 12). Early stages in the failure are characterised by the appearance, at fairly regular intervals, of steeply-inclined, oblique splits in the walls (Plate 4, fig. 19). These splits gradually widen and as the shearing continues the tangential walls of the fractured cells become separated, the shreds produced elongating by stretching (Plate 2, fig. 12, Plate 4, fig. 19). This stretching of the shreds is accompanied by the appearance on them of fine parallel lines which are inclined to the direction of the original splits.* The shearing in the substance of the walls of the tracheides usually takes place independently of the bordered pits and is often confined to a very narrow zone of the wall near to the corner of the tracheides. The bordered pits remain intact, and instead of forming places of weakness in relation to the longitudinal shearing, appear rather to be a source of strength to the walls. It seemed possible that the shreds seen in the specimens fractured in the tangential direction might be derived in part from the tangential walls of the tracheides as well as from the radial walls shown in Plate 2, fig. 12, and Plate 4, fig. 19. Transverse sections of fractured specimens, however, proved conclusively that when the shearing takes place in the tangential plane, only the radial walls of the tracheides are shredded, and the tangential walls are not visibly affected by the stress (Plate 2, fig. 14).

When the wood of Spruce is subjected to longitudinal shearing along a radial plane (text-fig. 8), the stress operates both on the autumn and spring wood and affects each differently. In the spring wood, as before, the fracture occurs by a regular shredding of the walls along steeply inclined planes (Plate 2, fig. 13). Slight splits which are steeply inclined appear at fairly regular intervals; these gradually widen giving rise to shreds which elongate by stretching. This elongation of the shreds is accompanied, as before, by the appearance of fine parallel lines which are differently inclined from the splits (Plate 4, fig. 20).

In the autumn wood the fracture occurs by an abrupt separation of the radial walls at the middle lamella of the tracheides and at the medullary rays (Plate 4, fig. 21). There is, therefore, no shredding evident where the fracture passes through the autumn wood. The different appearances of the fracture, in the region of the autumn and spring wood respectively, are best seen in transverse sections through the fracture (Plate 4, figs. 21 and 22). The difference in the behaviour of the autumn wood is probably related to the greater thickness of the secondary layers of the cell-walls causing these to offer greater resistance to shearing than the middle lamella and the walls of the cells of the medullary rays.

The staining effect, obtained with chlor.-zinc-iodide in the case of compression failures, was also obtained in these shear failures. The shreds of the walls give the

* These fine parallel lines have all the appearances of the striations described by NÄGELI, CORRENS, etc., for the walls of fibres and other plant cells, but as is pointed out below, no such striations have ever been observed in the present work on wood, except in relation to mechanical injury and in the wood near branches.

blue colour similar to that obtained in the zone of failure in compression, and the striæ on the shreds appear as dark lines of a deeper blue colour. The mechanical stress of longitudinal shearing, therefore, produces the same sort of fundamental change in the staining properties of the cell-walls which has already been described for compression failures.

In addition to this staining effect the fractures in longitudinal shearing show features which can be further correlated with the characteristic method by which failure is initiated in compression. The longitudinal shearing stresses, operating on the wall of a tracheide (if this were isotropic), would tend to produce separation along two systems of planes inclined approximately at 45° to the direction of stress. The actual result on the thin-walled tracheides is consistent with this expectation except for the inclination of the planes. The oblique splits represent the direction of one of the series of planes of separation, while the fine striæ on the shreds are slip-lines due to the stretching. In some few instances splits occur simultaneously in both series of planes of separation giving an irregular fracture such as is seen in Plate 4, fig. 23. Much more often the splits are confined to one of the series of planes of separation and shreds are produced which show striæ or slip-lines. The anisotropy of the cell-wall probably explains the fact that the inclination of the planes of separation, as manifested by the splits and the striæ on the shreds, is not 45° to the direction of the stress.

The inclination of the planes of separation as seen on the surfaces of the walls, unlike that of the gliding planes, which have been decribed above, for compression failures, is similar to, or identical with, that of the slits of the pits. This correspondence between the inclination of the splits and that of the pit-slits, as well as the possible nature and effect of the natural anisotropy of the cell-wall will, be discussed below, in connection with the general questions bearing on the internal structure of the woody cell-walls.

Discussion of Results and Theoretical Considerations.

The discovery that the permanent deformation of wood under mechanical stress takes place by the development of planes of slipping in the walls of the cells of which it is composed, has led to fundamental conclusions regarding the ultimate mechanical properties of wood. The properties depend primarily on the behaviour of the substance of the cell-walls, and only secondarily on the arrangement of the individual cells and tissues in the wood. In this sense wood is a mechanical structure the qualities of which depend even more on the material of construction, that is the cell-wall substance, than on the distribution of material in the structure.

The behaviour of the cell-wall substance towards mechanical stresses in the development of planes of slipping, presents striking similarities to the mechanical properties of other substances. Reusch (*loc. cit.*), in 1867, described the production of gliding planes in the crystals of many ordinary salts as a result of compression. His

observations have been extended by many other investigators, and the gliding planes are now recognised as localised planes of slipping which may form planes of cleavage for the crystals. More recently EWING and ROSENHAIN (*loc. cit.*) have shown that the plastic deformation of metals is due to the development of gliding planes within the crystals of which the metals consist. They hold that plastic deformation can only take place by gliding on the slip-planes of true crystals, and that the plasticity of metals is bound up with their crystalline structure. The slip-planes arise in directions which bear a definite relationship to the crystallographic axes, but as far as I am aware no relation has been traced between the directions of stress and those of the slip-planes in the individual crystals of the metals.

The well-known LÜDER'S lines which often appear on strained specimens of certain metals have, however, according to HARTMANN* and also to GULLIVER,† inclinations which approximate to those expected of planes of maximum shearing, *i.e.*, 45° to the direction of the stress. It is also generally recognised that LÜDER'S lines are outward manifestations of large numbers of the microscopic gliding planes in the crystals of the metal. The general direction of the slip follows the plane of least resistance in the substance of the metal, since it is reasonable to suppose that the minute size of the heterogeneous crystalline components allows the metal to behave as an isotropic substance.

The substance of the cell-walls of wood is probably, most correctly, regarded as of a colloidal nature, but at the same time it must be pointed out that the cell-walls show many important resemblances to crystals. These resemblances must be taken into account in the consideration of the nature of the planes of slipping which arise in wood under mechanical stress.

NÄGELI‡ was the first to attempt to relate the properties of cell-walls to conceptions of molecular physics. He studied the double refraction of cell-walls, their swelling properties and the visible microscopic structure, and was led from his observations over a wide field to propose the micellar hypothesis of the structure of cell-walls and other organised substances. According to this hypothesis the cell-wall consists of crystalline molecular aggregates or micellæ, arranged in a definite manner to build up micellar complexes. The micellar complexes are orientated in rows, and the fine spiral striations seen on the walls of many elongated cells represent alternating micellar rows having respectively greater or less water-content. The inclination of the striations, when present, is the same as the inclination of the slits of the pits, and even where striæ are not readily visible the direction of the micellar rows

* GULLIVER, "Some Phenomena of the Permanent Deformation of Metals," 'Proc. Inst. Mech. Engineers,' 1905, p. 141 ; 1907, p. 579.

† HARTMANN, 'Distribution des Déformations dans les Métaux, etc.,' Paris, 1876.

‡ NÄGELI, 'Bot. Mitth.,' vol. 1, "Die Anwendung des Polarisation apparates auf die Unters. des vegetabilischen Elementartheile," 1862, p. 183 ; 'Bot. Mitth.,' vol. 2, "Über den innern Bau der vegetabilischen Zellenmembranen," 1864, pp. 1–102 ; 'Theorie der Gährung,' 1879.

has been inferred by NÄGELI and later investigators from the slits of the pits. NÄGELI found that the directions of greatest swelling and of greatest optical elasticity were connected with the direction of inclination of the striæ. In the elongated cells of wood the axis of greatest swelling is in a radial direction in the wall, that is at right angles to the surface. The axis of least swelling lies in the tangential plane, that is parallel to the surface of the wall, and is inclined in the spiral course of the striæ or pit-slits. An intermediate amount of swelling takes place along a tangential axis at right angles to this. The axes of optical elasticity are the inverse of these, the least in the radial direction, the greatest along the spirals of the striæ, and the intermediate one at right angles to these.

The main trend of botanical opinion has favoured, more or less completely, the micellar hypothesis of NÄGELI; it is a remarkable tribute to the hypothesis that even quite recently some workers with colloids, such as BACHMANN,* VON WEIMARN,† and BRADFORD‡ should have made use of some of the main conceptions of NÄGELI, in explaining the structural properties of gels. It has, however, been frequently pointed out that many of the facts, on which the micellar hypothesis was based, are incorrect. These facts need not be referred to in detail, but NÄGELI'S conception of essentially crystalline character of the cell-wall in plants must be considered in relation to the present work on wood. To-day, the substance of the cell-wall is usually regarded as an emulsoid gel. It is, therefore, probably non-crystalline in structure for, according to WO. OSTWALD§ and to BACHMANN, no emulsoid gel has been proved to be crystalline. VON WEIMARN, however, regards many gels as possessing crystallinity, and BRADFORD has recently extended VON WIEMARN'S theory to certain of the organic gels. Alternative explanations to the micellar hypothesis have been proposed, but, with the possible exception of STRASBURGER'S‖ molecular-net hypothesis, none of them has attempted to explain more than one or two of the sets of properties first studied (in this connection) by NÄGELI. PFEFFER¶ has pointed out that the properties of elasticity and rigidity have not been investigated in connection with the molecular structure of organised bodies, and I am not aware of any previous work from this point of view. The bearing of the behaviour of the cell-wall substance of wood towards mechanical stress, on hypotheses of the structure of cell-walls must, therefore, be dealt with at some length.

Striations of the nature of those described by NÄGELI (*loc. cit.*), CORRENS,** DIPPEL,††

* BACHMANN, "Unters. u. die ultramikros. Struktur von Gallerten," 'Zeit. für Anorgan. Chem.,' vol. 73, p. 128 (1912).

† WEIMARN, VON, 'Koll. Zeitschr.,' vol. 4, p. 317 (1908); vol. 5, p. 62 (1909); vol. 6, p. 32 (1910).

‡ BRADFORD, S. C., 'Biochem. Jl.,' vol. 11, 1917; vol. 12, 1918; 'Science Prog.,' vol. 12, 1917.

§ OSTWALD, WO., 'Colloid Chemistry' (Eng. Ed.), 1915, pp. 56–65, etc.

‖ STRASBURGER, 'U. d. Bau Wachstum d. Zellhäute,' 1882; 'Histologische Beiträge,' vol. 2 (1889).

¶ PFEFFER, 'Plant Physiology,' vol. 2, pp. 70–83.

** CORRENS, 'Jahrb. für Wiss. Bot.,' 1892, p. 254; 1894, p. 587.

†† DIPPEL, 'Das Mikroskop,' 1898.

KRIEG* and others have not been observed in the woods here investigated when in the uninjured condition. But this is not surprising, since the descriptions referred to are for the most part from wood obtained in the vicinity of branches or from fibres which had been subjected to chemical or mechanical treatment. In the specimens used in the present work, there was little possibility of any of the pieces consisting of wood from the neighbourhood of branches.

Although, however, striæ have not been met with, spirally inclined slits of pits have been observed continually on the walls of the tracheides and fibres (Plate 1, fig. 9). It has been mentioned above that the inclination of these slits has usually been regarded as a good indication of the direction of the micellar rows.

It has been shown in the above study of the failure in longitudinal compression of Spruce, Ash, and Pitch Pine, that the planes of slipping are always inclined differently from the planes of inclination of the slits of the pits, and generally form a high angle with them. In Spruce, for example, the slits of the pits are steeply inclined, and only form a small angle with the axis of the cell, as seen in surface view. The slip-lines run transversely across the surface of the walls of the thin-walled cells or are inclined at a high angle (more than 45°) in the thick-walled cells. In both cases the lines run obliquely in the section of the wall, but at an angle of more than 45°, to the long axis of the cell.

The displacement for compression failures is thus never along the planes of the slits of the pits, and, except for the fact that the inclination of the slip-planes to the direction of stress is always greater than 45°, the behaviour of the material of the walls is such as might be expected from an isotropic substance.

The anisotropy of the cell-walls, as regards their mechanical properties, is more striking in connection with the different behaviour in tension and compression. It is well known that the strength of wood is much greater in longitudinal tension than in end-compression. The different microscopical characters of the fractures can be correlated with this difference. It has been seen that increasing stress in end-compression leads to the development of increasing numbers of slip-planes in the substance of the walls, but that actual rupture rarely, if ever, occurs along these, failure being finally manifested by buckling and crinkling of the fibres. In tension there is a greater resistance to the development of slip-planes, and very few of these appear, but slipping along them is followed by rupture almost immediately. The microscopic facts, therefore, indicate that the gradual plastic yielding of the substance of the cell-walls, which occurs in compression, does not take place to anything like the same extent in tension. The fact that, in Spruce, after the initial ruptures have occurred in tension, the rest of the material fails by longitudinal shearing, does not affect this conclusion. If the substance of the cell-walls were isotropic, it seems

* KRIEG, 'Beihefte zum Bot. Centralb.,' vol. 21 (1907).

reasonable to suppose plastic yielding would be manifested both in tension and compression in a similar manner.

In tension the inclination of the planes of slipping differs for different woods, but two main types have been observed. These are illustrated by normal Spruce on the one hand, and by Pitch Pine, brittle specimens of Spruce, and by Ash on the other. In the first of these types the slip-planes follow the same inclination as the slits of the pits. The hypothetical micellar rows, therefore, form planes of weakness for longitudinal tension in the cell-walls of normal Spruce and Ash. In the second type* (*e.g.*, Pitch Pine, brittle Spruce, and Ash) the slip-planes are orientated in the same manner as in the compression failure, and, consequently, in these cases, the planes parallel to the slits of the pits do *not* form planes of less resistance than the rest of the wall. In the first type there seems to be less cohesion, for tension stress, between the particles of wall-substance along the planes of the slits, than in any other direction. In the second type there is no such obvious difference between the cohesion in any one direction and in any other,† and the fracture therefore approximates more closely to planes of maximum shear in the cell-walls. It is perhaps noteworthy in this connection that brittle specimens of Spruce often give strength-values in tension tests which are not so widely different from the values in compression tests as are those of normal Spruce.‡

The failure of Spruce in longitudinal shearing is initiated by the appearance of obliquely-inclined splits, on the surface of the walls, which stand at right angles to the plane of shearing. The natural anisotropy of the cell-wall causes the splits usually to follow the inclination of the slits of the pits; the shreds produced, elongate by slipping, along planes inclined to these. The slip-lines on the shreds present all the characters of the spiral striations described by NÄGELI and his followers for the walls of many elongated cells.

When longitudinal shearing stresses operate on thick-walled cells, *e.g.*, in the autumn wood of Spruce, or in the fibres of hard woods, separation occurs in the middle lamella between adjoining cells. The middle lamella offers less resistance to the shearing stresses than the other layers of the thick wall. This mechanical weakness of the middle lamella is not manifested in the tension and compression failures since the stress is mainly taken by the other layers of the wall in these cases.

It would be possible to use, to some extent, the facts described above in support of the micellar hypothesis of NÄGELI. It has been shown that, in regard to their mechanical properties, the cell-walls of wood manifest an anisotropy closely parallel to that shown in regard to such other physical properties as double refraction and swelling. In terms of the micellar hypothesis, the axes of greatest optical elasticity,

* Andaman Padouk and Swamp Cypress also conform to this type.

† The different degrees to which plastic yielding by slipping occurs in tension and compression, respectively, of course holds for both types.

‡ I am indebted to Major ROBERTSON, R.A.F., for this information.

of greatest mechanical rigidity, and of least swelling on the one hand, and the axes of greatest swelling, of least mechanical rigidity, and of least optical elasticity on the other, would be identical. This would be in general agreement with the facts derived from the microscopic appearances of the cell-walls of wood after failure in compression, in tension and in shearing in a longitudinal direction respectively. It has already been pointed out, however, that the conception of crystalline micellæ as the ultimate particles of cell-wall substance is not in accord with many modern views regarding the structure of colloid gels (p. 71). Neither is the micellar conception necessary to explain the facts described above for wood. It seems to me that a mechanical hypothesis of the structure and properties of the colloidal cell-wall explains all the known facts.

The salient points of the alternative hypothesis of cell-wall structure, which I have been led to propose from this study of the minute mechanical properties of the cell-walls of wood, will now be outlined. The cell-wall in plants has usually been regarded as a secretion product of the protoplasm. On the basis of modern views of surface energy and adsorption, it is conceivable, however, that the membrane in vegetable cells arises, first, by the accumulation of pectic substances, and then of cellulose, at the surface bounding the protoplasm. This accumulation at the surface would occur passively, as the substances arose in the protoplasm by reason of their capacity to reduce the surface energy of the protoplasm according to the GIBBS-THOMPSON law. The cellulose, thus accumulating at the protoplasmic boundaries in the growth of the membrane, will be in the condition of a highly viscous fluid, but, as the membrane grows, the viscosity increases, and finally the membrane attains the qualities of a rigid gel.

In the flow of mobile liquids, the displacements of the particles in relation to one another are uniform, in other words, such liquids under mechanical stress exhibit homogeneous shearing. EWING and ROSENHAIN (*loc. cit.*) have further pointed out that even viscous fluids may manifest homogeneous shearing. KUNDT,* however, has shown that, when even dilute sols of gum, collodium, etc., are subjected to mechanical deformation by rapidly rotating layers between two glass surfaces, a transitory double refraction is observed. The shearing produced in such cases must be heterogeneous, but, since no microscopic change is visible, an ultramicroscopic heterogeneity of shear must be thought of in this case. If, however, a membrane possessed even a small amount of rigidity, the shearing produced under mechanical deformation might be permanent, and yet ultramicroscopic, and thus only manifested by the development of double refraction. On the other hand, the permanent deformation of a more rigid membrane would give rise to a more gross heterogeneous shearing along microscopic planes of shear, the traces of which would be visible in the membrane. In other words, in the shearing of mobile, viscous, semi-viscous, and rigid substances, there must be every transition possible, from a condition

* KUNDT, 'Wied. Ann.,' vol. 3, p. 110 (1881).

of absolutely uniform or homogeneous shearing of the particles, through a permanent ultramicroscopically heterogeneous shearing, to a grosser heterogeneity of shearing in rigid substances. In such rigid substances, the individual planes of shear are separated by apparently homogeneous layers, with no visible manifestation of shearing.

From the above theoretical considerations, it follows that the anisotropy of the cell-wall can be explained as due to strains* produced by the operation of mechanical stress on the semi-rigid substance of the cell-wall in its developmental history. In other words, in the growth and solidification of the cell-wall, large numbers of minute slip-planes develop, and this causes the wall to become anisotropous. These planes are either wholly ultramicroscopic, or also microscopic in character; in the one case they are manifested only by the double refraction of the cell-wall, and in the other also by the striations and the slits of the pits. It is perhaps not without significance, in relation to this hypothesis, that the young cell-wall, *e.g.*, of cambium cells, is not doubly refractive, but only becomes so later. BUTSCHLI† has shown that it is possible, by applying tension to hardened threads of gelatine, to produce markings which present extraordinary similarity to the fine striations of bast fibres. Although BUTSCHLI did not apply a mechanical significance to these markings, which I also have observed, it seems to me there is no doubt that they represent planes of slipping in the threads. It is further noteworthy that it is quite easy to produce striations in cell-walls, artificially, by mechanical treatment, and the conclusions of NÄGELI and others are open to criticism on this account. Such artificial "striations" have been described above in connection with the failure of the wood of Spruce in longitudinal shearing. NÄGELI regarded his mechanical treatment as merely revealing the predetermined inner structure of the wall. In my view, many of the striations were created by this mechanical treatment, and really represent microscopic planes of slipping in the wall-substance.

Little is known of the forces available in the growing cells of plants to account for the permanent deformations postulated above. It is, however, well known that in the development of tracheides and fibres from cambium cells considerable extension both in length and in diameter of the cells takes place. It is true that NÄGELI and SCHWENDENER‡ have maintained that this extension takes place by growth and not merely by stretching, but STRASBURGER,§ KRABBE‖ and others have shown that plastic stretching often occurs. The mechanism by which such plastic stretching could

* The strains, thought of, are real strains in the mechanical sense, and not the internal tensions or stresses postulated by VON HÖHNEL and STRASBURGER, and recently revived by HARRISON ('Roy. Soc. Proc.,' A, vol. 94, 1918) to explain the double refraction of textile fibres.

† BUTSCHLI, "Unters. über Strukturen, u.s.w.," 'Verh. Nat.-Hist. Verein Heidelberg,' vol. 4 (1896).

‡ NÄGELI and SCHWENDENER, 'Das Mikroskop,' 1877, 2nd Aufl.

§ STRASBURGER, *loc. cit.*

‖ KRABBE, "Beitrag zur Kenntnis der Struktur u. des Wachstums Veg. Zellhäute," 'Jahrb. Wiss. Bot.,' vol. 18, pp. 346–424 (1887).

take place would be the development of the ultramicroscopic or microscopic slipping conceived of above.

In the present work it has been shown that permanent deformation in the cell-walls of wood takes place by the development of planes of slipping in the substance. This conception is now being carried back to the developmental history of such cell-walls and the peculiarities of the natural walls are regarded as manifestations of mechanical forces which have operated on them during the course of their development. In this connection it is perhaps significant that in the vast majority of the cases where striations have been observed the cells have an elongated form. It is further possible that some of the mechanical forces acting on the cell-wall in the course of its development arise as the hardening membrane contracts by loss of water in its fixed position.

In wood the greater resistance to plastic deformation by slipping in tension than in compression would receive some explanation on the view that tensile strain in the previous history of the cell-walls had rendered them less susceptible to further plastic deformation in tension. It is conceivable that further slipping in tension is not possible because all the available planes of slip have been utilised for the developmental straining. ROSENHAIN (*loc. cit.*), BEILBY,* and others have described similar phenomena for metals. These often show hardening and enhanced resistance to plastic deformation in tension after having undergone tensile strain. In some such cases the strain-hardened metal is more easily deformed in compression than in tension: that is, the strain hardening is uni-directional. This mechanical anisotropy in strain-hardened metals is somewhat analogous to that seen in wood.

The analogy, with the essentially crystalline metals, must not be pressed too far for wood, which, on the the views already expressed is not truly crystalline in nature; but the resemblances may not be wholly without significance. In the case of wood it is held that the planes of slipping are not orientated in relation to any real crystallographic axes, but that they approximate to the planes of maximum shearing according to the direction of the stress. The orientation of these planes, however, is never 45°, as for isotropic substances, but is modified for particular stresses by the previous plastic deformations the wall-substance has undergone in the developmental history of the cell-walls. The above explanation of the mechanical anisotropy of the cell-walls of wood in my view, also satisfactorily explains the anisotropy in regard to their other main physical properties. The hypothesis that the mechanical anisotropy, as well as the double refraction and visible structure of the cell-walls may be explained as a result of mechanical causes operating on the substance of the cell-wall in the course of its development from a highly viscous fluid to a more rigid condition, has been framed almost solely in relation to facts derived from the study of the mechanical properties of wood. Further detailed investigation of the properties of the cell-wall

* BEILBY, 'Roy. Soc. Proc.,' A, vol. 72 (1903); vol. 76 (1905); vol. 79 (1907); vol. 82 (1909).

in plants over a wider and more varied field will show whether the hypothesis can have a more generalised application.

Summary.

1. The initial phenomenon of the permanent deformation of wood under end-compression, longitudinal tension, and longitudinal shearing, is the development of microscopic planes of slipping in the substance of the cell-walls of the wood.

2. The gross characteristics of failure in compression are described for Spruce, Ash, and Pitch Pine. The broad results for Ash and Pitch Pine confirm those of previous investigators, but Spruce shows important differences. It is shown that the gross characters of the failure in different woods are probably determined by the anatomical structure of the wood, but that primary changes due to the development of planes of slipping in the cell-walls precede the secondary effects described by previous investigators. The microscopic planes of slipping have not previously been recognised in wood.

3. The development of the slip-planes in the cell-walls is accompanied by profound changes in the behaviour of these towards many stains and reagents. The altered parts of the cell-walls behave as if free cellulose were present there. This effect is discussed in relation to its possible bearing on the process of lignification of cell-walls.

4. The gross and microscopic features of the failure of Spruce, Ash, and Pitch Pine in longitudinal tension are described. In all cases failure is preceded by the development of slip-planes, but only relatively few of these arise, and rupture occurs along some of them much more quickly than in compression failures.

5. The failure in longitudinal shearing is described for Spruce, and it is shown that the manner of failure is to some extent affected by the relative thickness of the cell-walls under stress. Failure takes place by the development of slip-planes, if the walls are relatively thin, and by separation at the middle lamella if the walls are thicker.

6. An attempt has been made to correlate the microscopic effects of the various forms of stress with the visible structure of the cell-walls, and with the double refraction of these. It has been found that the slip-planes developed in compression failures are not inclined similarly to the planes of the slits of the pits on the cell-walls. The failure in tension of normal Spruce, and also in longitudinal shearing of the spring wood of Spruce, occurs along planes having the same inclination as the slits of the pits, and therefore the same as that of the hypothetical micellar rows of Nägeli. In many other woods, the failure in tension occurs along slip-planes similarly orientated to those obtained in compression failures.

7. The general behaviour of the cell-walls of wood is discussed in relation to the micellar hypothesis of cell-wall structure. The microscopic characters, accompanying the permanent deformation of wood by tensile and compressive stresses respectively, are different. The differences, which consist in the much smaller amount of slipping

which precedes rupture in tension than in compression in all the woods studied, and in the different inclinations of the planes of slipping in some of the woods, can be correlated with the differences in the numerical values, obtained for the strength under these stresses. It is pointed out that this mechanical anisotropy of the cell-walls is not wholly inconsistent with the micellar hypothesis of NAGELI, which was framed to explain the anisotropy in other properties of cell-walls. Since, however, fundamental portions of NÄGELI'S hypothesis, such as the postulating of crystalline ultimate particles, are not necessary to explain the facts, an alternative hypothesis of cell-wall structure is proposed.

8. In the hypothesis, it is suggested that the mechanical anisotropy, as well as the double refraction and visible structure of cell-walls, may be explained as a result of mechanical causes operating on the substance of the cell-wall in the course of its development from a highly viscous fluid to a more rigid condition.

DESCRIPTION OF PLATES.

PLATE 1 (Photographs).

s., spring wood; *m.r.*, medullary ray; *r.*, place of rupture; *x.*, slip lines; *a.*, autumn wood; *f.*, zone of failure; *m.l.*, middle lamella; *p.s.*, pit-slits.

Fig. 1.—Compressed piece of Spruce, showing the zone of failure as seen on the tangential face. × 5¼.

Fig. 2.—The same piece of Spruce as in fig. 1, showing the zone of failure as seen on the radial face. × 5¼.

There is marked displacement in the radial direction; the displacement is most apparent at the limits of the annual rings, *i.e.*, where the autumn wood of one year abuts on the spring wood of the next year. There is no such evident displacement on the tangential face (fig. 1).

Fig. 3.—Tangential longitudinal section of a compressed piece of Pitch pine, showing the steeply inclined zone of failure. The displacement in the tangential direction is very marked, and an incipient zone of failure is seen in addition to the main zone. × 28.

Fig. 4.—Portion of the zone of incipient failure from the same section as fig. 3, showing the separation between the tracheides and the medullary rays. × 67.

Fig. 5.—Radial longitudinal section of compressed Spruce. A portion of a zone of failure is seen, showing the crinkling and buckling of the tracheides of the spring and of the autumn wood respectively. The radial displacement is obvious, and some separation of the elements has taken place at the annual ring. × 67.

Fig. 6.—Tangential longitudinal section of a compressed piece of Ash, showing the inclined zone of failure. The displacement in the tangential direction and the separation between the fibres and the medullary rays are clearly seen. × 28.

Fig. 7.—Tangential longitudinal section passing through the zone of failure in Spruce. × 9.

This has been stained by chlor.-zinc-iodide, and the reagent has swollen the zone of failure and straightened the tracheides. It shows the remarkable alteration in the staining properties of the zone of failure.

Fig. 8.—Radial longitudinal section passing through the zone of failure in Spruce. × 9.

This section was similarly treated to that in fig. 7, and shows equally clearly the change in the staining properties of the zone of failure. The reagent has swollen the zone and straightened the tracheides so that the displacement in the radial direction is not obvious, but the staining brings out the peculiar outline of the zone of failure in relation to the annual rings.

Fig. 9.—Portion of radial longitudinal section through compressed Spruce, showing the early stages in the development of the zone of failure. The bands of failure appear light on the darker background.

The crinkling of the spring tracheides is seen and also the early stages which lead to buckling in the thicker-walled autumn wood. The inclined slits of the bordered pits are seen in the photograph. × 67.

Plate 2 (Photographs).

Fig. 10.—Tangential longitudinal section of a compressed piece of Spruce, showing early stage in failure by crinkling of the walls of the tracheides. × 67.

Fig. 11.—Radial longitudinal section of a compressed piece of Spruce, showing slightly later stage in failure by crinkling of the walls of the tracheides. × 67.

Fig. 12.—Radial longitudinal section of a specimen of Spruce, fractured by longitudinal shear in a tangential plane. × 67.

The shearing has occurred in the walls of tracheides of the spring wood, and the wall substance has been stretched out into obliquely running shreds. (See also Plate 4, fig. 19.)

Fig. 13.—Tangential longitudinal section of a specimen of Spruce, fractured by longitudinal shear in a radial plane. The shearing has taken place in the walls of the tracheides, and the incipient production of shreds is seen. × 67.

Fig. 14.—Transverse section of similar piece, fractured by longitudinal shear in a tangential plane, to that shown in fig. 12. × 67.

The drawing out into shreds is seen to be confined to the substance of the radial walls of the tracheides.

Fig. 15.—Portion of radial longitudinal section passing through marginal portion of zone of failure in Spruce treated with chlor.-zinc-iodide. × 67.

The deep stain within the zone of failure is seen to be due to the multiplicity of bars of wall-substance which have stained selectively. These bars extend beyond the actual zone of failure, but are much less frequent, being separated by considerable zones of unaltered cell-walls.

Fig. 16.—Portion of tangential longitudinal section similar to the tangential section shown in fig. 15. × 67.

Again the presence of the deeply staining bars is seen, even outside the actual zone of failure.

Fig. 17.—Walls of tracheides of autumn wood of Spruce, in radial longitudinal section, showing commencement of permanent deformation by the development of oblique planes of slipping in the secondary layers of the wall-substance. No slip lines are evident in the middle lamella.

Photographed by polarised light with crossed nicols. × 350.

Fig. 18.—Similar photograph to fig. 17, showing the multiplication of slipping planes leading to crinkling of the cell-walls. × 350.

Plate 3 (Drawings).

(Figs. 1 to 7 of Spruce.)

Fig. 1.—The walls of the autumn tracheides of Spruce seen in tangential longitudinal section, showing very early stages in the development of slip planes. No bending has yet occurred. × 900.

Fig. 2.—Walls of two adjoining tracheides of autumn wood similar to those in fig. 1, showing later stages in the slipping. The number of planes of slipping has greatly multiplied, and buckling is about to occur. The step-like projections of the substance of the wall are shown. × 900.

Fig. 3.—Walls of tracheides of autumn wood of Spruce, showing still later stage than in figs. 1 and 2. Buckling has now taken place as a result of the great multiplication of lines of slipping. × 600.

Fig. 4.—Walls of tracheides of spring wood of Spruce in radial section, showing early stage in the development of slip-planes, and in this case rapidly leading to crinkling. × 900.

Fig. 5.—Walls of tracheides of spring wood of Spruce at slightly later stage than in fig. 4, showing crinkling due to the multiplication of slipping planes. × 900. (*Cf.* Plate 2, fig. 11.)

Fig. 6.—Walls of tracheides of Spruce in radial section, showing oblique slipping lines both in the thickness of the tracheides and on the surface of the walls. The lines on the latter are not inclined at the same angle as the slits of the pits. × 275.

Fig. 7.—Walls of tracheides of spring wood of Spruce from same radial section as fig. 6, showing obliquity of slip-planes in the thickness of the walls and the horizontal character on the surface of the walls. × 275.

Fig. 8.—Walls of the tracheides of autumn wood of Pitch Pine in tangential longitudinal section, showing multiplicity of shearing planes leading to buckling. × 600.

Fig. 9.—Walls of fibres of Ash, showing multiplicity of shearing planes leading to buckling. × 900.

Fig. 10.—Walls of autumn tracheides of Spruce, fractured in tension, showing fibrous character of the break owing to shearing having occurred both in the radial and tangential walls. The inclination of the planes of fracture is approximately that of the slits of the pits. × 600.

Fig. 11.—Walls of tracheides of spring wood of Spruce in same specimen as fig. 10, showing similar oblique planes of fracture. An incipient fracture is also seen developing along similarly inclined planes at S. × 600.

Plate 4 (Drawings).

Fig. 12.—Walls of tracheides of autumn and spring wood of a brittle piece of Spruce, showing tension fracture spreading across them. × 80.

Fig. 13.—A few of the walls shown in fig. 12. Rupture has occurred in the spring tracheides, and the development of a very few slip-lines is seen in the autumn tracheides. × 275.

Fig. 14.—Walls of tracheides of autumn wood of Spruce, showing the horizontal break on surface of wall and oblique break in the thickness of the wall along slip-planes. The inclination of the slits of the pits is indicated. × 600.

Fig. 15.—Fractured ends of fibres of Ash. The break is along the oblique slip-planes, which are not inclined at the same angle as the pits. × 275.

Fig. 16.—Tracheides of Pitch Pine, fractured in tension. The break has occurred along slip-lines which are developed also in the vicinity of the rupture. × 600.

Fig. 17.—Walls of tracheides in fractured tension specimen of Pitch Pine, showing slip-lines. A bordered pit is shown, and this clearly has not proved a source of weakness. × 600.

Fig. 18.—Tension fracture of Ash (somewhat diagrammatic). The break has had no obvious relation to the medullary rays. × 80.

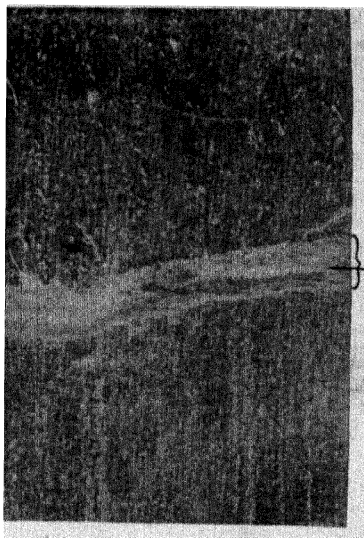
Fig. 19.—Radial longitudinal section of a specimen of Spruce, fractured by shearing along a tangential plane. The shredding of the walls is seen and also the fine slip-lines on some of the shreds. The bordered pits are intact, and only a narrow zone of the wall is stretched out—*cf.* width with that of the adjoining tracheide. × 275. (*Cf.* with Plate 3, fig. 11.)

Fig. 20.—Walls of tracheide from fractured region of a specimen sheared in the radial plane. Stretching of the wall has occurred with incipient shredding and the development of fine lines of slipping. × 275.

Fig. 21.—Transverse section through autumn wood of fractured specimen sheared in radial plane. The separation has occurred at the middle lamella. × 275.

Fig. 22.—Transverse section through the spring wood of the same specimen as in fig. 21, showing the drawing out of the tangential walls into shreds. × 275.

Fig. 23.—Walls of tracheide from fractured region of specimen sheared in the tangential plane. Separation is taking place along two series of obliquely inclined planes. × 275.

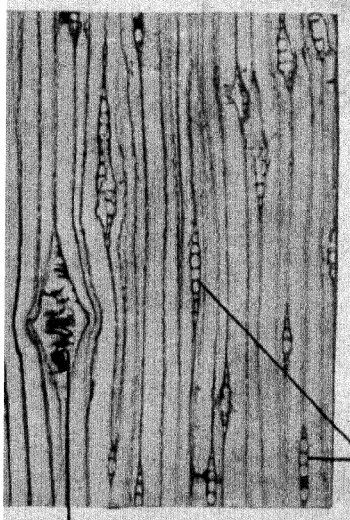


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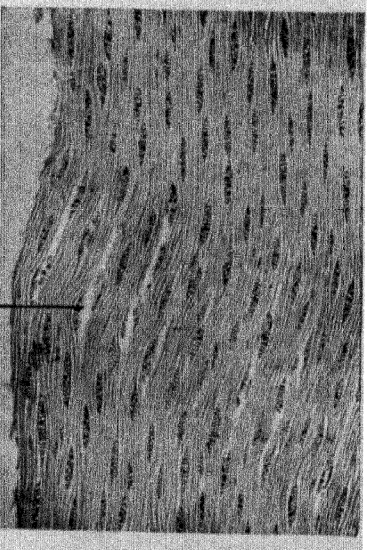
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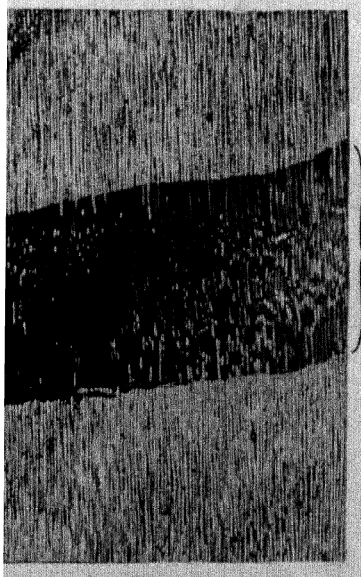
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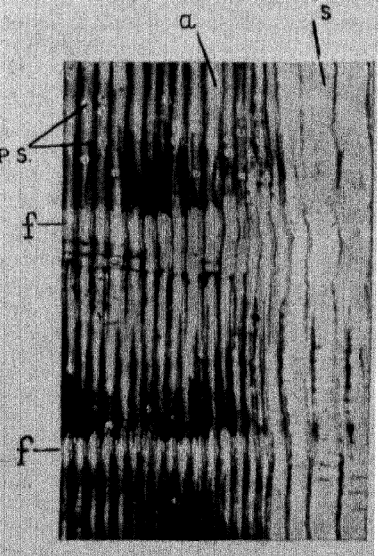
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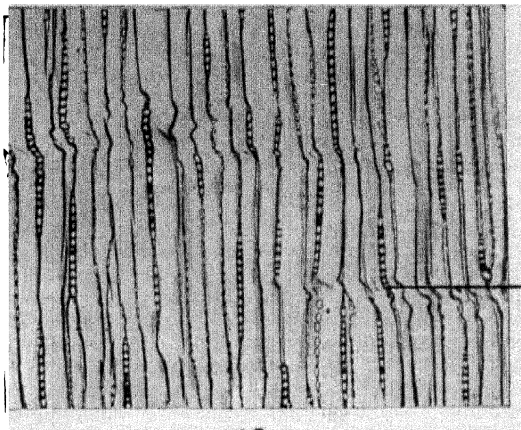
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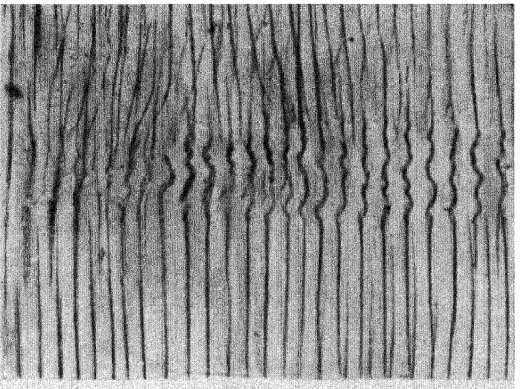
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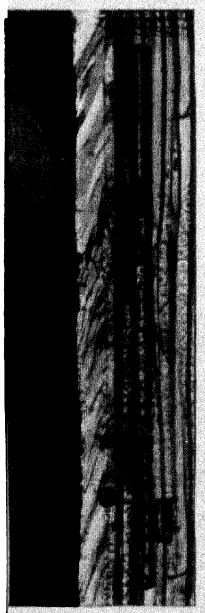
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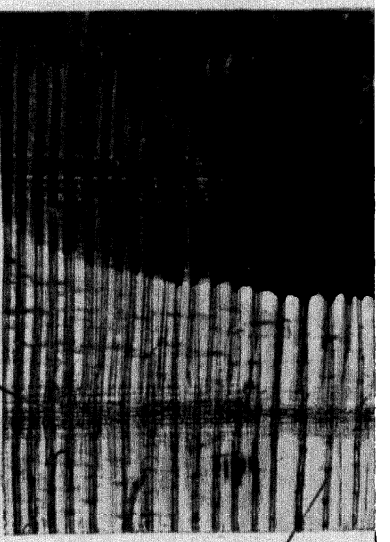
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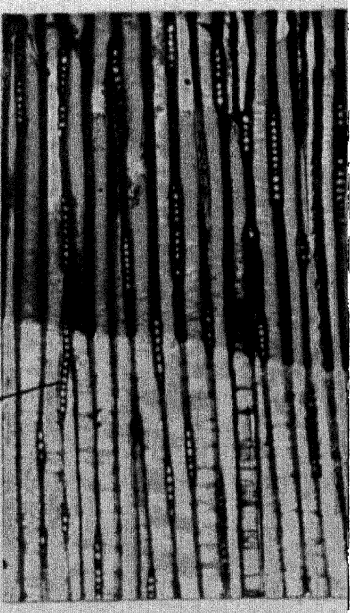
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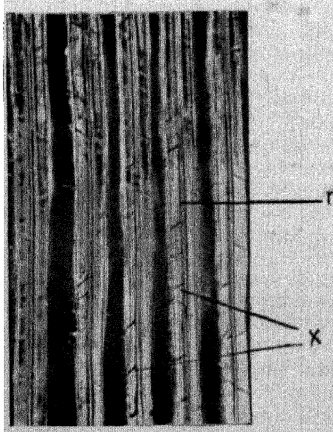
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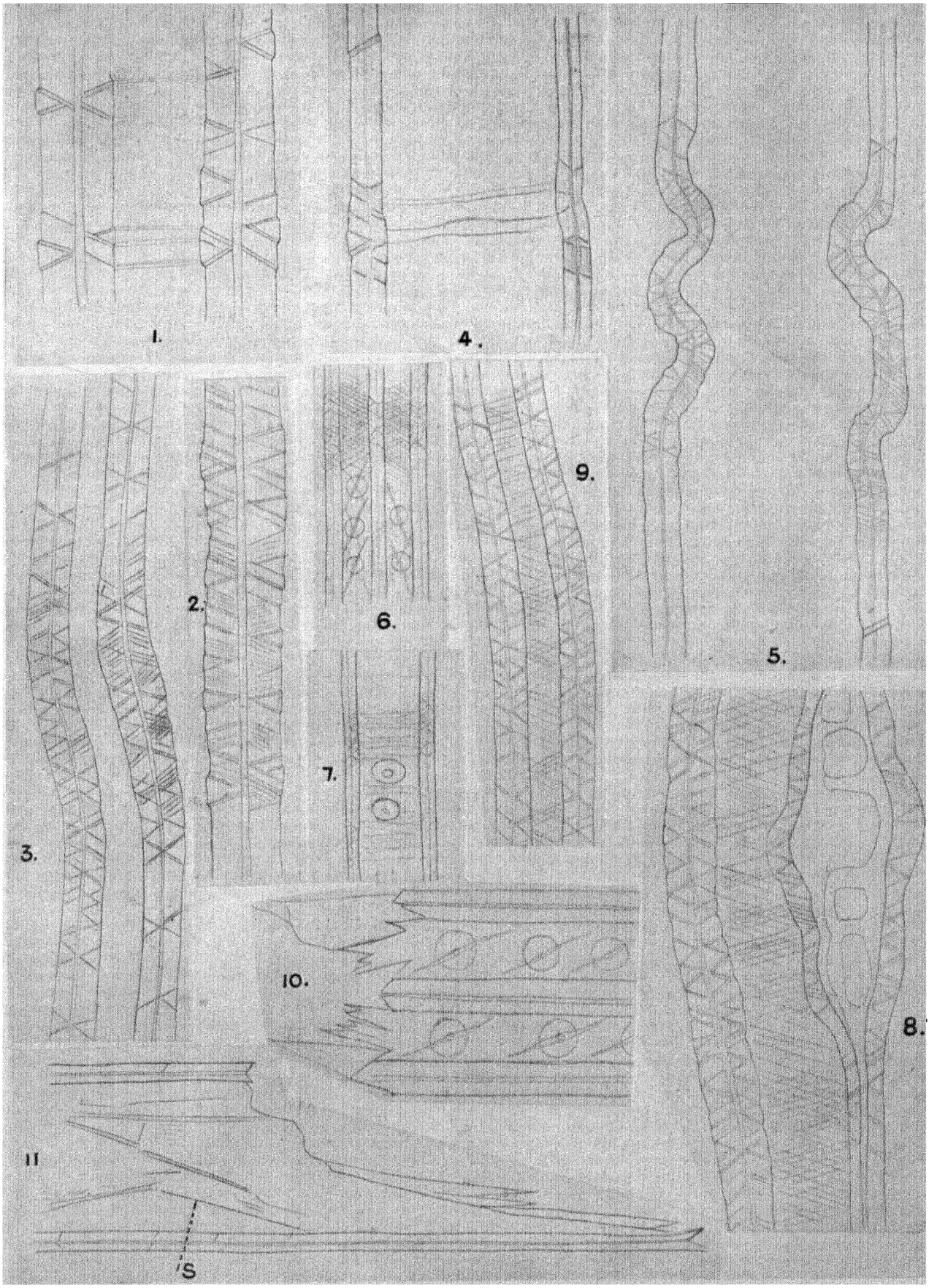
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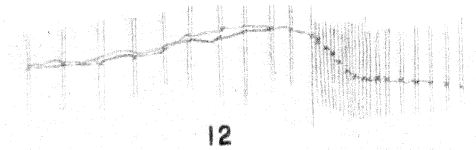


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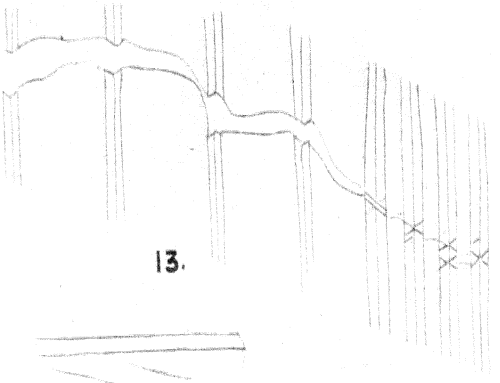


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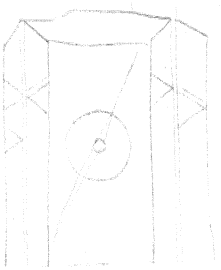
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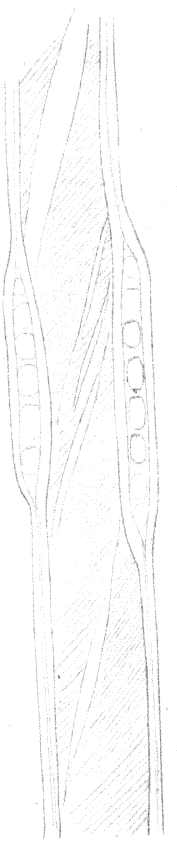


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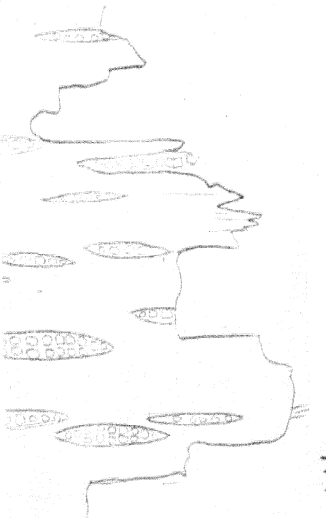
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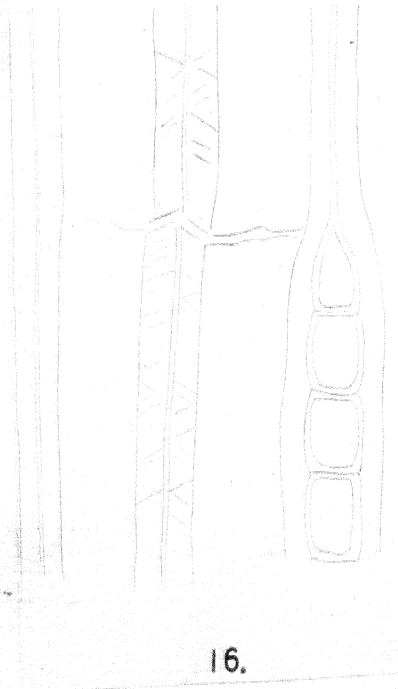


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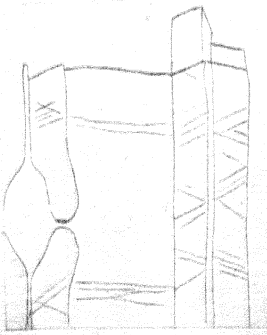
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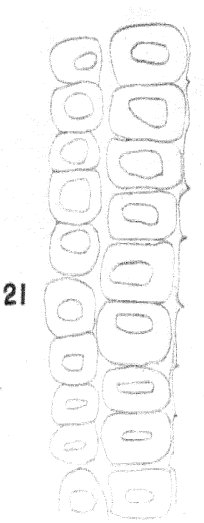
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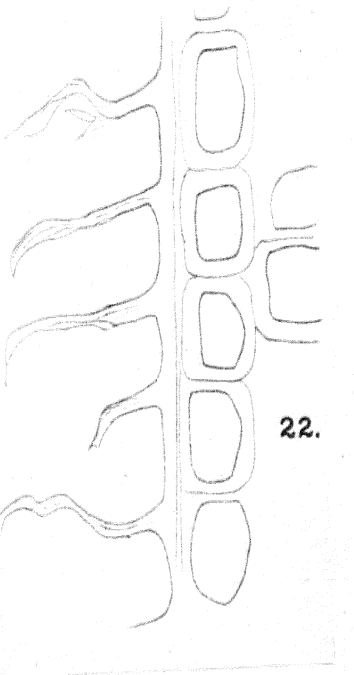
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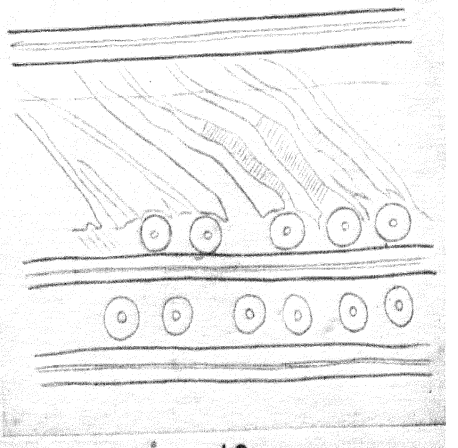
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III.—*On a Form of* Botrytis cinerea, *with Colourless Sclerotia.*

By William B. Brierley (*Rothamsted*).

Communicated by Dr. E. J. Russell, *F.R.S.*

[Plate 5.]

(Received January 31, 1920,—Read March 18, 1920.)

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1. Introduction.

Botrytis cinerea is perhaps the commonest and best known fungus, and has been a centre of mycological research since the time of de Bary. Few, if any other fungi, have been studied so thoroughly by so many able investigators, or are the subject of so extensive a literature; and one need only draw attention to the researches of de Bary (6), Pirotta (61), Marshall Ward (80), Kissling (45), Nordhausen (58), R. E. Smith (70), Farneti (27), Beauverie and Guilliermond (9, 10), Istvanffi (33), Reidemeister (65), and the more recent researches from the laboratory of Prof. V. H. Blackman (13, 16, 17, 84). There is a body of experimentally ascertained and exact knowledge concerning the bionomics of this fungus, which can be exceeded by that of few other micro-organisms.

For the particular purposes in view in my investigations, it has been imperative

that all experimental work be carried out with pedigree cultures. It was very early discovered that the ordinary "pure-culture" of mycological and bacteriological laboratories may be and very often is a poly-genotypic population, and that very considerable genotypic differences may usually be found in apparently homogeneous populations exhibiting only one single type, around which the individuals fluctuate. In order to eliminate this vitiating factor, single-spore cultures were prepared either by BURRI's (19) Indian-ink method, plate isolation, or, more usually, by a combination of the plate and dilution-drop method. These cultures served as the initial source of experimental material. In all, over seventy such pedigree stocks have been prepared from original sub-strata, representing about sixty different species of host plant, derived from all parts of the country. From these stocks some fifteen thousand cultures have been made. During the course of the investigation, many of these have been subjected to the most diverse environmental conditions, and all have been maintained under the closest scrutiny. With the exception of the single culture to be described, no change that could be interpreted as a permanent heritable alteration or mutation has been observed.

2. LIFE CYCLE OF *Botrytis cinerea*.

In nature, the fungus usually appears as a delicate smoke-grey velvety pile, covering diseased plant tissues or organic *debris*, or, on more resistant sub-strata, in the form of isolated grey pustules. This superficial growth consists of the branched conidiophores bearing spores. On germination, which under favourable conditions occurs in a few hours, each of the latter gives rise to a mycelium, which in two or three days produces a further crop of conidiophores and spores. When old, the hyphæ and spores may produce microconidia, which germinate directly giving rise to mycelium. If at any stage of development conditions unfavourable to sporogeny intervene, the mycelium produces sclerotia, and these, under more favourable conditions, give rise to tufts of conidiophores bearing spores. Under certain adverse conditions, the hyphæ may produce chlamydospores, or themselves break up into oidia, and these, on germination, give rise to normal mycelium.

It was the opinion of DE BARY (5, 6), VIALA (79), ZOPF (89), and other early investigators, an opinion based purely on superficial resemblances and the frequent contiguity of growth of the organisms, that *Botrytis cinerea* is only the conidial phase of a Discomycetous fungus, which was referred to either *Sclerotinia Fuckeliana* or *S. libertiana*. In the absence of any proof of this connection, and despite their own negative experience, this opinion was accepted by MARSHALL WARD (80), KISSLING (45), and others, and so became an integral part of mycological literature. In 1905, ISTVANFFI (32) published a voluminous memoir, purporting to bring forward proof of the genetic relationship of *Botrytis cinerea* and *Sclerotinia Fuckeliana*. In spite of the prior and much more incisive work of R. E. SMITH (70), this evidence has been generally accepted. It is, however, of

doubtful value, for a critical examination of the investigation makes clear that ISTVANFFI has confused two distinct fungi, which possess certain superficial resemblances, often grow together on the same host, and are not easily separable in culture.

Recently, SEAVER and HORNE (68) claim to have established the relationship of an unnamed species of *Botrytis* with a new species of *Sclerotinia*, which they have termed *S. Geranii*. The evidence is very brief, and awaits confirmation, and, in view of the fact that many "pure-cultures" of fungi are undoubtedly mixed populations, that *Botrytis cinerea* and various species of *Sclerotinia* and other genera harmonise perfectly in their growth when developing intermixed, both on natural hosts and on many artificial media, and that, unless specially searched for, the very minute microconidia of both forms are easily overlooked, and may act as a contaminating factor, one may perhaps hesitate to accept unreservedly this evidence. Furthermore, in my own study, some fifteen thousand cultures of various strains of *Botrytis cinerea* and nearly related "species" have been closely observed under the most varied environmental conditions, and much experimental work has been specially directed towards the elucidation of the genetic relationships of the fungus. I have, however, found no evidence indicating that *Botrytis cinerea* is in any way a developmental phase within the life-cycle of *Sclerotinia Fuckeliana* or genetically related to any other species of this genus. Repeatedly, strains of *Botrytis cinerea* and various species of *Sclerotinia* have been grown side by side by inoculating alternate quadrants of a plate culture, and, under all conditions, have remained separate. My experience merely confirms that of LIND (47), R. E. SMITH (70), PELTIER (59), PETHYBRIDGE (60), and others, who have paid special attention to this aspect of the problem.

Botrytis cinerea as a discrete entity is an asexual fungus, and the critical importance of a *Sclerotinial* relationship lies in the possibility which this introduces of a sexual process. If the organism be sexual, any single individual may possibly be heterozygous, and there is then no inherent improbability that segregation may occur, resulting in the appearance of apparently new forms which might mistakenly be interpreted as mutants.

In many *Mucorineæ*, *Chytridineæ*, *Saccharomyces* and possibly other *Ascomycetes* the sexual process is allogamous and factorial segregation is not impossible. In the great majority of fungi, however, in which sexuality occurs, and here is included so far as is known the entire group *Discomycetineæ*, which contains the genus *Sclerotinia*, the process is autogamous. Now it is well known, and has recently been mathematically demonstrated by JENNINGS (35), that even in an originally heterozygous organism, self-fertilisation if continued generation after generation leads rapidly to a condition in which the offspring are homozygous. In such case JOHANNSEN (36, 37) has shown that the mere isolation of the progeny from a single reproductive member produces a pure line.

Even, therefore, in the remote contingency that *Botrytis cinerea* is merely a developmental phase of *Sclerotinia Fuckeliana* and that this possesses a sexual process—which has yet to be demonstrated for any species of this genus—it would appear that the complications resulting from heterozygosis are absent from our problem. For critical purposes *Botrytis cinerea* is, on all evidential criteria, an asexual homozygotic organism in which the isolation of a single spore strain necessarily implies the isolation of a "pure-line." A genotypic change in a pure line is a mutation.

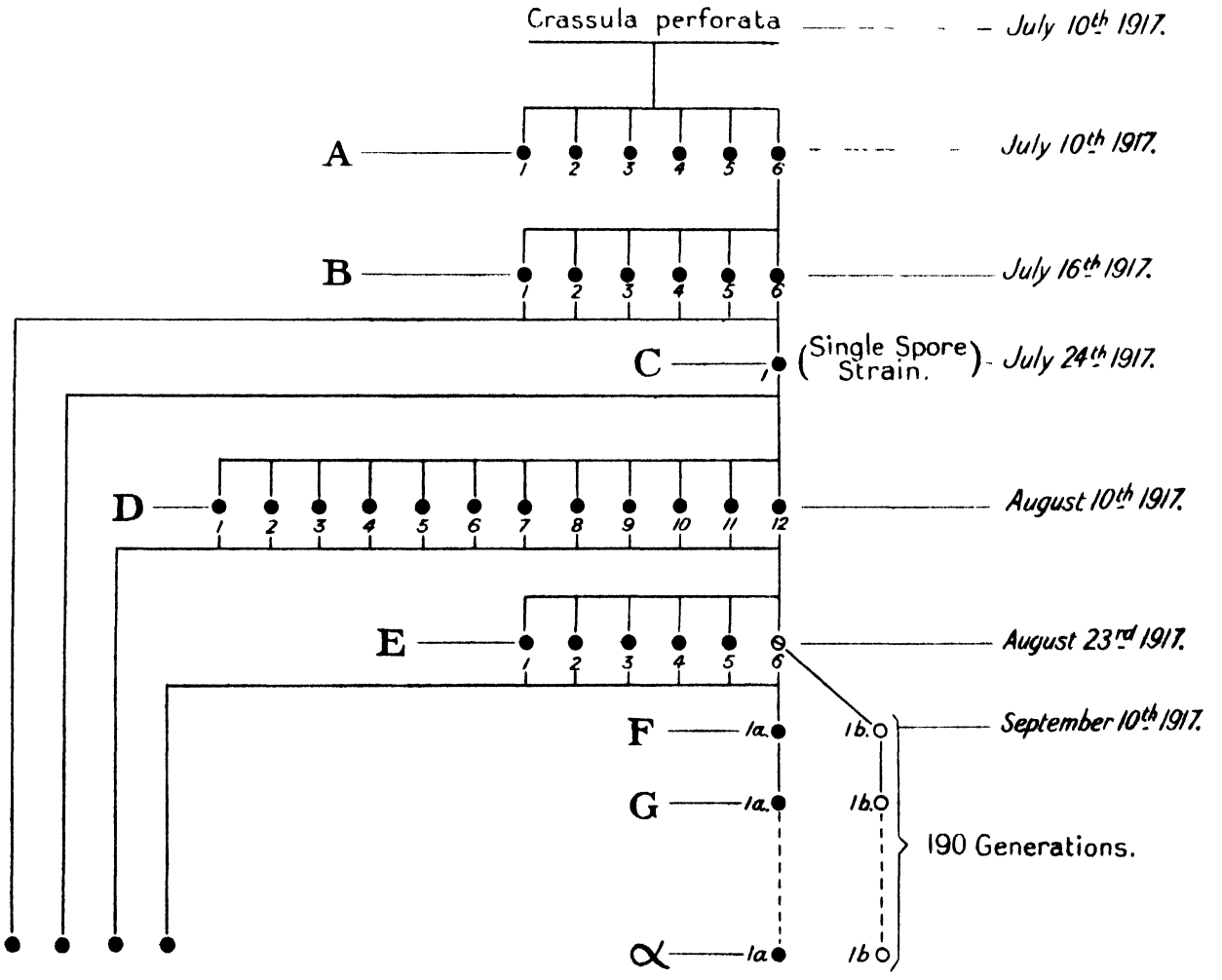
3. Origin of Colourless Form. General.

On July 10, 1917, a diseased specimen of *Crassula perforata* from the succulent house of the Royal Botanic Gardens, Kew, was examined, and the causal pathogen identified as *Botrytis cinerea*. The growth was normal in every respect, and from it six cultures, (A. 1–6), were prepared. These appeared to be free from contamination by other organisms, and on July 16, six further cultures, (B. 1–6), were taken from (A. 6). On July 22 all twelve were carefully examined and found to be free from contamination. A series of dilution drops were then prepared from (B. 6), many of these drops containing but a single spore. The latter were transferred to a thin plate of potato-agar and their development scrutinised carefully during two days. A colony whose origin from a single spore had been ascertained with certainty was then picked up on a platinum wire and planted in a tube slant. The spores are comparatively large, the most common size on the host plant being $7{\cdot}5\ \mu \times 9{\cdot}5\ \mu$; they are thus easily seen and isolated. On germination they become still more obvious, and the obtaining of a single-spore strain is thus a simple procedure. On August 10 twelve cultures (D. 1–12), were prepared from the pedigree stock (C. 1), and on August 23 six further cultures, (E. 1–6) were made from (D. 12). So far the work had merely been a part of the ordinary laboratory routine, the cultures which were all on tube slants of potato-agar being used for various experimental purposes.

About a week later cultures (E. 1–6) were examined and some three or four days later re-examined. The six cultures had all been placed together in a wire basket and left on the laboratory bench. It was noted that in tube (E. 6) one of the sclerotia had not turned black like the others but had remained colourless. In certain strains of *Botrytis cinerea* these bodies do not form the black pigment characteristic of the genus until they are mature, and although this particular sclerotium differed from its fellows in apparently following this mode of behaviour, it was merely regarded as a somewhat youthful individual, perhaps a little unusual, but not particularly noteworthy. On September 10, however, when tubes (E. 1–6) were again examined and the aberrant sclerotium in (E. 6), now quite mature, was found to be still lacking any sign of pigmentation, it was decided to isolate this sclerotium and breed from it. In tube (E. 6) there were in addition to the colourless sclerotium 24 normal black sclerotia and a black sclerotial crust around the bottom of the tube, where the medium had contracted away from the glass.

The colourless sclerotium was removed and thoroughly washed in sterile distilled water to remove adherent spores. It was then broken open and a fragment from the centre taken out, placed on a tube-slant of potato-agar (F.I. *b*) and incubated at 22° C. A vigorous growth ensued and a week later sclerotia began to be formed. In the tube there were ultimately formed 17 sclerotia and all were colourless.

TABLE I.



In a normal sclerotium the pigmentation is confined to the outer one to three layers of cells (text-fig. 1) and as it is from these that the conidiophores arise on germination, it was considered not impossible that these cells and their derivatives only might carry the power of colour formation. If, therefore, a fragment of the inner hyaline tissues of such a normal sclerotium were planted on a nutrient medium it seemed possible that colourless sclerotia similar to those obtained in tube (F.I. *b*) might develop. Accordingly, a black sclerotium from (E. 6) was treated thus—exactly as the aberrant sclerotium had been treated. The subsequent culture (F.I. *a*) contained uniformly black sclerotia.

There were now in existence two distinct strains (F.I. *a*) and (F.I. *b*) of the fungus

both derived from a single-spore stock (C. 1). Of these the parental form (F.I. *a*) possessed the highly characteristic black sclerotia; whilst the new strain (F.I. *b*) which had arisen from this apparently by a single direct saltation, possessed colourless sclerotia. The history of the two forms is shown in a diagrammatic manner in Table I. The distinguishing lettering (A. 1–6) and so forth, and the arrangement of descent in this Table have been adopted for the sake of clearness in the account. In practice (D. 1–12) were merely 12 similar cultures taken from (C. 1), and (E. 6) was taken from one of these cultures chosen at random. In the same way six cultures were made on July 16 and from one of these chosen at random (C. 1) was prepared.

It seemed possible that an albinistic tendency might be a " weakness " inherent in this particular race of *Botrytis cinerea*, and that if the preceding generations were further subcultured colourless sclerotia might again appear. Unfortunately the original diseased plant and the first six cultures made from it had been destroyed, but all subsequent cultures had been retained. As no particular care had been taken of these some had become contaminated. In all cases, however, it was possible to subculture purely from them and this was done extensively for many generations under the most diverse conditions of light, temperature, food supply and so forth, all the resulting cultures being most carefully examined for any indication of a lack of sclerotial pigmentation. All were perfectly normal.

The parental strain and its colourless derivative have each, up to the time of writing, passed through over two hundred direct tube generations with very many lateral subcultures, and although these have been placed under diverse environmental conditions each strain has remained absolutely constant.

Since this colourless strain arose, many thousands of cultures and fresh specimens of *Botrytis cinerea* have passed under my observation, but in no single instance have I found any similar absence of sclerotial pigmentation. Prof. H. H. Whetzel, of Cornell University, who for some years has been engaged upon a monograph of the genus Botrytis and has received and compared material from many parts of the world, informs me, after having examined a culture of the colourless strain, that he has " never seen anything quite like it." In the voluminous literature devoted to the fungus there is no record of a form with colourless sclerotia.

Having regard, therefore, to our knowledge of the fungus *Botrytis cinerea* and to the particular conditions under which the aberrant strain originated, it would appear difficult on present criteria to interpret it as other than a true mutation.

4. Comparison of Parental Strain and its Colourless Derivative.

The obvious difference between the original and the derived forms lies in the colour of the sclerotia. Mutations in the fungi *Aspergillus niger* and *Penicillium glaucum* have been described at length by Arcichovskij (2), Waterman (83) and Schiemann (69). In these cases, however, although the obvious change was in spore

colouration, there were other less visible changes in several independent respects, certain morphological characters and physiological activities of the derived fungi being different from those of the original forms. In addition to these facts, the results of recent researches on the linkage of characters and the multiple allelomorph interpretation of quantitative inheritance made it appear probable that in the present case the colour change might be only one of many related changes less obviously visible.

Moreover, whether one regards the black sclerotial pigment as a mere excreted product or as an integral and actively functioning substance in the metabolic activities of the organism, the pigment itself is the result of a long and elaborate series of causally dependent processes, and a sudden and permanent loss of the power to excrete or form such matter can only be the visible expression of deep-seated physiological changes in the developing organism. But a living organism is an extremely complex and delicate equilibration of rhythmic metabolic processes in a colloid substratum, and it is difficult to conceive of any sudden derangement of these activities which has not reverberations in many directions.

It seemed, therefore, very desirable to make some comparison of the parental strain and its colourless derivative to ascertain the nature of any changes other than pigmentation which might be present. This comparison proceeded along several lines, the main directions of which are indicated briefly below.

A. *Physiological Activities.*

If, as evidence would seem to show, the physiological reaction of a particular organism is constant only in an unvarying environment, it follows that identity of response to like stimuli implies identity of constitution, and, conversely, that a change in physiological constitution will be reflected in a changed reaction to unaltered conditions. Only if this is true may a comparison of the physiological activities of two organisms be instituted; and being true, it will afford the most delicate test of individual or genotypic identity, for the technique is quantitative and the nicest differences may be measured.

(a) *Germination of Spores.*—The effect of the character of the nutrient medium upon the germination of fungus spores is often very marked, and not infrequently this relation may be used to differentiate two or more genotypes otherwise difficult to distinguish. The percentage germination within certain periods of time when the spores were immersed in distilled water, 15 per cent. gelatine in water, and Coons' solution (21) were compared in the two experimental forms and in two strains used as a control. The spores were taken from three-day cultures on potato-agar incubated at 22° C., and the hanging drops in which the germinations were tested were maintained in the cool incubator at 18° C. The results are shown below, the numbers representing percentage germinations.

Medium.	Parental Strain.			Colourless Strain.			Control Strain A.			Control Strain B.		
	6 hours.	12 hours.	24 hours.	6 hours.	12 hours.	24 hours.	6 hours.	12 hours.	24 hours.	6 hours.	12 hours.	24 hours.
Distilled water	10	27	55	12	25	53	33	51	68	25	55	93
15 per cent. gelatine	43	67	93	41	66	90	47	78	90	47	72	90
Coons' solution	63	83	97	61	80	96	63	90	98	61	72	98

The lethal temperature of the spores was tested by making spore suspensions in 15 per cent. gelatine in distilled water and heating corresponding tubes of parental, derived, and control strains together in a water bath for ten minutes, the temperature rising by increments of five degrees. The experimental strains both gave growth in tubes heated to 45° C., but not in those heated to 50° C., whilst in the control tubes growth was inhibited by temperatures of 45° C. and 55° C. respectively.

(b) *Growth on Nutrient Media.*—One of the most critical and easily determined measures of the identity of two fungi is their growth and behaviour upon various standardised nutrient media under controlled conditions. The general characters of the colony, the colour reactions of hyphæ and medium, and the many other detailed phenomena which may be observed are valuable diagnostic characters, and sharply reflect the physiological differences separating two genotypes. At the end of his paper on "Cultural Studies of Species of *Penicillium*," THOM (77) has drawn up a scheme of comparative cultural data, which, in the present absence of any more detailed formulation, could with valuable results be adopted by mycologists as a basis of systematic diagnosis. In the comparative examination of the experimental strains this technique was followed, and the results compared with those of two control strains. No essential difference could be detected between the former, but these stood in marked contrast with the latter. For laboratory purposes other than this particular investigation, the parental strain and its colourless derivative were largely used in experiment, so that the cultural comparison of the two forms was amplified in very many ways. In all cases where they were grown under parallel conditions no essential difference could be detected. The comparative development of the two strains and control cultures was tested further at various temperatures, and with different intensities of light and conditions of aeration, but the results obtained only confirmed those referred to above.

A few qualitative experiments were carried out to test the comparative enzyme production of the fungi, the methods used being adapted from those described by CRABILL and REED (22) in 1915. On starch-agar the experimental strain and one control showed approximately equal amylolytic action under the colonies, while the second control showed a distinct halo indicating the presence of a diffusible extra-

cellular amylase. These differences were thrown up more clearly when the plates were flooded with iodine solution. On litmus-cream-agar growth of the experimental strains was profuse, and there was strong acid production, the red colour diffusing through the medium. In both control strains the lipolytic action was equally marked but the redness much more sharply defined. All the four strains liquefy gelatine and slowly dissolve fibrin in fibrin-agar. In both experimental strains the lipase production is feeble and only slight browning of the fibrin occurred, this being markedly different from the results in the controls. On casein-agar moderate growth occurred, and ereptic action was prominent, extending in a distinct band around the colonies. The production of erepsin was distinctly greater in both controls than in the experimental strains. Sparse development occurred on asparagin-rosolic-acid-agar with slight amidase production. The red colour in the experimental strains was less diffuse than in the controls.

Cytase formation was tested by growing the organisms in Dox's solution (23) the carbon source being filter paper. Moderate growth occurred, but after a fortnight there was only a barely perceptible reduction with FEHLING'S solution in the experimental strains and one control strain, whilst the second control showed more distinct cytolytic action. Calcium-carbonate-agar prepared with DOX'S solution (23) plus one per cent. of lactose showed a feeble though distinct production of lactic acid, this being more marked in the experimental than in the control strains. In all the experiments no essential differences in the enzymic activities of the normal strain and its aberrant form could be detected.

(c) *Pathogenicity.*—Marked and constant differences in virulence for different hosts are shown by the various strains of *Botrytis cinerea.* In testing the comparative pathogenicity of the experimental strains, the spores, together with a fragment of potato-agar, were planted on corresponding surfaces such as the opposite sides of a fruit, or opposite halves of a leaf. On banana, apple, and tomato fruits, lily and potato leaves, tulip bulbs, and twigs of horse-chestnut infection readily occurred, and vigorous development ensued. On onion-bulb scales, potato tubers, crab-apple, cucumber, and fruits of *Pyrus japonica*, leaves of *Primula sinensis* and wall-flower, infection occurred with very considerable difficulty and only when the tissues were badly bruised, and the growth tended to die out rapidly. Bulbs of snowdrop and leaves of Portuguese laurel and rhododendron could not be infected. In no case was any essential difference in pathogenicity noted between the parental and colourless strains. Markedly different results were obtained with the two control strains, of which one was originally derived from onion bulbs and the other from fruits of *Pyrus japonica.*

The cultural examination of the parental strain and its colourless derivative, although put separately above, was only part of a much larger series of experiments which were being carried out in the laboratory to ascertain the physiological differences of closely similar morphological strains, and the identity of result obtained

in this comparison very largely takes its critical value from its relation to this wider issue. Briefly, it may be stated that so far as the physiological constitutions of the parental strain and the colourless strain may be judged from their behaviour, the two strains are identical.

B. *Morphological Characters.*

Phenotypic plasticity is shown by most fungi, and is not only evident in those general characters which together constitute the morphological facies of the organism, but in those critical structures, the reproductive bodies, whose constancy has so often been assumed.

(d) *Dimensions of Spores.*—During recent years it has been shown in a general way by many students of the fungi, notably perhaps by STEVENS and HALL (76), and following their investigations by ELLIOTT (26), MOREAU (53), MUTTO and POLLACCI (57), GÄUMANN (30, 31), and others, that the size of fungus spores is a function of the particular organism and the environmental conditions. With regard to the present fungus, *Botrytis cinerea*, under certain standardised conditions, the spore dimensions are a function of the particular pure line and the quality of the nutrient substratum ; and for any particular pure line, different standardised food-media give different but mathematically constant quantities for the modal values of the variation curves of the spores. On a series of standardised media under constant conditions, therefore, the modal values of the spores of any pure line of the fungus may be represented by a curve which is constant and characteristic for that pure line. The critical quantity is not the extremes of variation, but the curve of modal values ; and, other factors not varying, a difference of genotype is immediately reflected in this curve of modal values.

The modal values of the spores of the parental strain and its colourless derivative were compared in this way, the number of measurements for each particular test being 500. The results of three such comparisons are shown on p. 93.

The conidiophores of the fungus are comparatively large and complexly branched structures, and the mechanical difficulties in the way of their minute comparison are such as to render it difficult to eliminate errors of selection. In so far, however, as these structures could be compared with regard to the length, diameter, manner of branching and abstriction of spores no differences could be distinguished between the normal strain and its aberrant form ; and this holds true also for the colour of the spore mass.

(e) *Haptera.*—One of the most interesting structural features of *Botrytis cinerea* are the organs of attachment, which were described by DE BARY (5, 6) under the name " Haft-organen," and are variously termed " haptera " or " appressoria."

Their presence or absence in a culture is determined by environmental conditions, and the controlling factors are different for the several strains of the fungus. The experimental strains behaved similarly in this respect, forming few haptera when

Medium.	Spore.	—	Parent.	Derivative.
Potato-agar	Length	Minimum	6·0 u	6·0 u
		Mode	9·5 u	9·5 u
		Maximum	12·0 u	12·0 u
	Breadth	Minimum	4·5 u	5·0 u
		Mode	7·5 u	7·5 u
		Maximum	10·0 u	10·0 u
Steamed potato	Length	Minimum	6·5 u	6·5 u
		Mode	10·5 u	10·5 u
		Maximum	16·0 u	16·5 u
	Breadth	Minimum	5·5 u	5·5 u
		Mode	9·0 u	9·0 u
		Maximum	13·5 u	14·5 u
Czapek's medium	Length	Minimum	6·0 u	6·0 u
		Mode	11·5 u	11·5 u
		Maximum	17·0 u	17·0 u
	Breadth	Minimum	5·5 u	5·0 u
		Mode	9·5 u	9·5 u
		Maximum	14·0 u	14·5 u

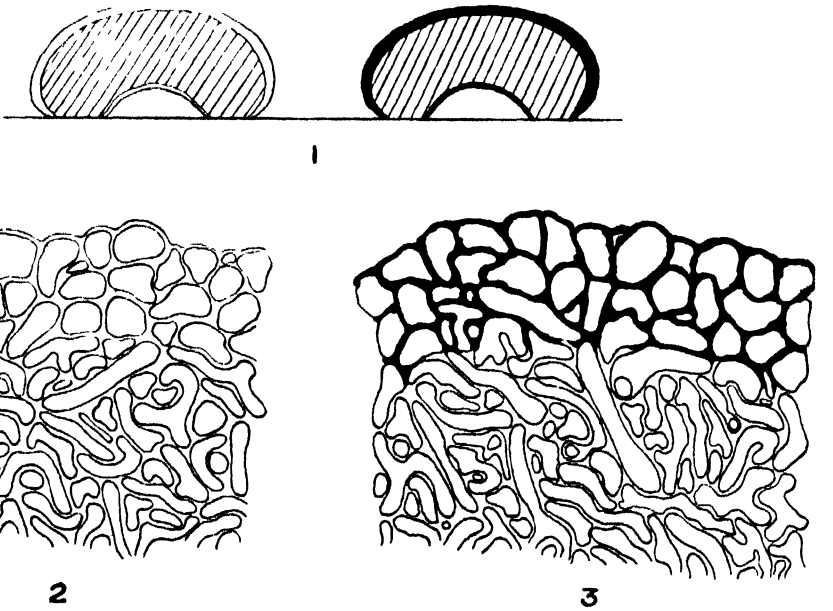
incubated at 22° C. on CZAPEK'S medium lacking carbon, producing them in abundance when M/10 glucose is added to this medium, and suppressing their formation when M/1 glucose is added. A similar course of behaviour is evident when the substratum remains constant and the temperature varies. The particular relations between hapteral formation and controllable external factors are very individual for the several strains of *Botrytis cinerea*, and constitute a diagnostic feature. The identity of behaviour between the normal strain and the colourless strain is therefore noteworthy.

(f) *Sclerotia.*—The type of correlation between hapteral formation and environment which has been indicated above holds true in the formation of sclerotia, and the presence or absence of these in any particular strain may be determined at will. It may briefly be stated that under all circumstances the modes of behaviour of the two strains with respect to this structure were alike.

Owing to the widely held belief regarding the constitutional weakness of albinos, as compared with the normal type, it was considered not improbable that the colourless sclerotia might show a lack of vitality and power to withstand adverse conditions. No indication of such weakness had appeared in the general cultural comparison of the two strains, but it seemed desirable to test the sclerotia themselves, for it is a common assumption that dark coloured or "carbonised" hyphæ are more resistant than hyaline hyphæ. The comparison was made by testing the relative germinative capacity of selected black and colourless sclerotia after subjection to various treatments. Parallel series exposed to extremes of temperature and desiccation for various periods exhibited no differential resistance, nor was this found on subjecting

the sclerotia to saturated atmospheres of chloroform and ether, or various dilutions of toxic substances such as mercury bichloride, copper sulphate, carbolic acid, and picric acid. Other strains used as controls gave markedly different results.

In structure the sclerotia vary according to the conditions under which they are formed. In contact with a hard surface they show an outer compact zone two or three cells in thickness which merges into an inner zone consisting of a firm colourless tissue-mass formed by closely interweaving hyphæ (text-fig. 1). On a soft matrix a third central zone of loose spongy hyphæ is usually present. The latter type of sclerotia are more or less spherical in shape whilst the former often fuse



TEXT-FIG. 1.

1. Vertical sections of colourless and normal sclerotia. Diagrammatic.
2. Vertical section of colourless sclerotium.
3. Vertical section of normal sclerotium.

} Swift ⅛ obj. No. 4 eyepiece.

together into a thin concave crust. The contact surfaces of both forms are colourless, but this is merely due to lack of aeration, for if the sclerotia be removed so that all surfaces have access to air, the previously colourless contact surfaces develop the tissue containing the characteristic pigment. If, however, the sclerotia be cut across so that the inner tissue zones are exposed, these do not develop pigment. The power of colour formation is therefore confined to the peripheral cells. Save for the absence of pigment in these cells, the most minute examination of the structure and form of the sclerotia of the parental strain and its aberrant form failed to reveal any difference.

Like the physiological examination the comparison of the morphological characters of the two forms under discussion has greatly increased value when it is realised that this work was only part of a wider series of experimental researches, which functioned as controls, and the results of which were invaluable in aiding in the

interpretation of doubtful phenomena. The conclusion drawn from the entire study was that the parental strain and the aberrant derivative differed in respect only to the one character of sclerotial pigmentation. The phenomenon would thus appear to form an exact parallel with mutations by loss of single genes in the higher organisms, and it would stand in contrast with the fungal mutations described by ARCICHOVSKIJ (2), SCHIEMANN (69), and WATERMAN (83), in which there was simultaneous variation in several independent respects.

The explanation of an apparent genotypic alteration on the basis of such a profound change as the sheer "dropping out" of an element from a most delicately balanced reaction-system would appear out of the question. I can only visualise the organism as a unity in itself, in the sense that it consists of a very great number of elements, each an elaborate reaction-system, which bear a specific and causal relationship to one another. The continued harmonious functioning of the total system minus one of its parts is to me unthinkable.

Pure lines of organisms are often separated by characters which are extremely minute and difficult to detect, as for example the obscure serological reactions which differentiate certain races of bacteria, and it would appear probable that were the analysis in the present case carried to a further degree of refinement, many deeply underlying and elusive physiological differences between the two strains would be detected. Such work, however, would constitute a most laborious and intensive study quite beyond the scope of the present paper.

5. ORIGIN OF COLOURLESS FORM. SPECIAL.

A question of some considerable importance is the exact point of origin of the colourless strain. As described it apparently arose in the single colourless sclerotium found in tube (E. 6), and for long no doubt was entertained that this was its actual point of inception. In the absence of further evidence, however, there was no certainty of this, for the colourless strain would only be observed on the production by it of sclerotia, and if sclerotia were not formed the presence of this strain could not even be suspected.

Cultures (D. 1–12) had been made from the pedigree stock (C. 1) in the usual way, by transference of spores on a platinum wire, and a large and indefinite number of these reproductive bodies would thus be implanted in each tube.

Approximately 100 per cent. of these spores would germinate, and the resulting growth would therefore comprise very many intermingled mycelia, some or all of which would produce conidiophores and spores. Similarly, the six tubes (E. 1–6) of August 13, prepared in the same way from one (D. 6) of the previous twelve, would each contain an indefinite number of mycelia, each mycelium corresponding to a single individual. Each tube might, therefore, contain one hundred or more individuals of identical genotypic constitution, growing inextricably mixed together and all or few producing spores and later sclerotia.

Furthermore, the first crop of spores produced from the mycelia in the tube may fall to the surface of the medium, immediately germinate, and two or three days later again reproduce, when the same process may be repeated. Thus in each *test-tube* generation there may be two or more *lineal* generations, depending partly upon the conditions to which the growth is subjected, and partly upon the available germinating surface of the medium.

From the pedigree stock (C. 1) to the culture of August 13 (E. 6) containing the first colourless sclerotium there were present three direct test-tube generations, which may actually consist of from about six to twenty lineal generations, and at some one point in this series of life cycles the aberrant strain may have arisen. In the series of cultures under consideration there was no evidence that any accessory spore form was produced, nor were the conditions such as would be conducive to their formation. A brief discussion of the two possible points of origin, the spore and the sclerotium, is perhaps desirable.

C. *Spore Formation.*

It will be clear that the colourless strain may have arisen as a variation in a single spore at any point between the second lineal generation in tube (C. 1) and the last lineal generation in tube (E. 6). If, for example, in the former, none of the offspring of this spore, although present in the later cultures, produced sclerotia until a favourable opportunity occurred in tube (E. 6). As, for essential purposes, the colourless form differs from the parental form only in the absence of sclerotial pigmentation, the presence of the former could not possibly be ascertained in a culture of the latter in the absence of the formation of its sclerotia. If, therefore, the aberrant form arose as a single spore variation, it is impossible to locate exactly its point of origin.

Certain facts relevant to the foregoing must, however, be noted. It has been an invariable technique throughout the whole of the research, when transferring inoculum from one tube to another, to take this either from a single conidiophore or, preferably, from a single germinated sclerotium. Now, every conidiophore and each sclerotium arises not only from a single original spore, but actually takes origin in a single mycelial cell. It will be obvious that this very considerably narrows the range wherein the aberrant form may have arisen as a spore saltation.

Furthermore, from its inception the colourless strain has been constantly reproduced by means of spores, and if it arose as a spore variation, the very existence of the new form implies that this particular spore germinated and produced a mycelium giving rise to conidiophores and spores, the latter possessing the new potentiality. But a single mycelium gives rise to an immense number of conidiophores, and the number of spores produced on even a single conidiophore is very great. It is, therefore, almost unthinkable that the new character should not again have appeared in any of the direct and lateral cultures made from tube (E. 6) and from the previous generations.

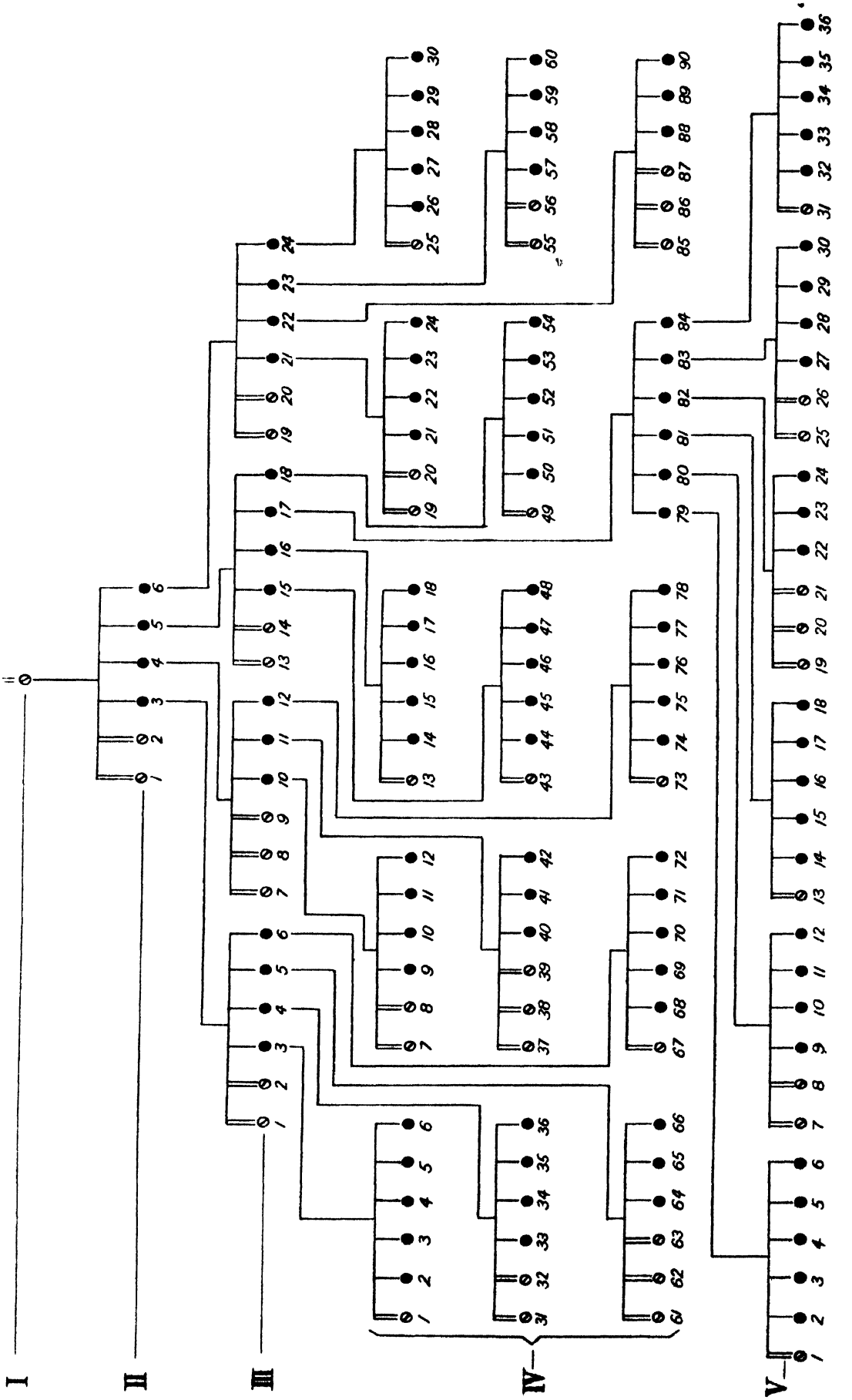
For purposes other than this particular piece of investigation it was decided to test the probability of the "throwing up" of the colourless strain in a parental culture, when only a single spore of the former is present. Accordingly, inoculum from (E. 4) was transferred to a clean tube-slant and streaked over the surface, after which there was inserted a single spore of the colourless strain. The circumstances would thus correspond in all essentials to those in tube (E. 6) if the aberrant form had arisen as a variation in a single spore. The results arranged diagrammatically are shown in Table II.

Tube (I) was prepared on March 2 and ten days later, when the sclerotia were mature, it was found that of the 23 sclerotia in the culture 2 were colourless, the aberrant strain thus "throwing up" in the first tube generation. On March 9 cultures (II. 1–6) were taken from (I) and ten days later it was found that 2 of these contained sclerotia of both strains, the remaining 4 containing only black sclerotia. In (II. 1) there were 15 black and 1 colourless sclerotium, and in (II. 2) 27 black and 1 colourless sclerotium. From each of the tubes containing only black sclerotia (II. 3–6) six cultures were made (III. 1–24). Of these 24 cultures 9 contained both parent and colourless form and the remainder black sclerotia only. In the former there were 162 black and 17 colourless sclerotia. From each of the 15 tubes containing only black sclerotia 6 cultures (IV. 1-90) were prepared. Of these, 24 tubes contained both black and colourless sclerotia in the proportion of 528 of the former to 39 of the latter, and the remaining 66 tubes contained only black sclerotia. In the 6 tubes (IV. 79–84), derived from (III. 17), only black sclerotia were present, and it appeared that in this line the colourless strain had "run out." To test if this were so, 6 cultures (V. 1–36) were made from each of these tubes. In at least 1 tube in each set of 6 the colourless strain was again thrown up, there being in all 10 tubes containing both kinds of sclerotia and 26 tubes containing only the black form. The work was not carried further than this; but in the total experiment, which represented 5 tube generations and 157 cultures, the aberrant form appeared in 46 tubes. A parallel control series prepared with similar inoculum from (E. 4) was synchronised with the foregoing, similar in all respects save that the single spore of the colourless strain was omitted. All the sclerotia were black.

It will be clear from this experiment that the presence of a single spore of the aberrant strain in a parental tube may cause the new character to appear in the first generation, and in a large proportion of cultures of succeeding generations. As already stated, the generations (C. 1), (D. 1–12), and (E. 1–6) were all subcultured freely, but the aberrant strain was absent from all descendants. In nearly 200 direct tube generations of the parent strain (F. 1*a*) the aberrant form has not appeared. This can only be explained by the hypothesis that the colourless strain did not arise as a spore variation, but arose in and was confined to the initial colourless sclerotium, and that the removal of this eliminated the aberrant strain from the parental culture (E. 6). The new strain was thus seen immediately on its inception and isolated.

TABLE II.

Inoculum from (E. 4) + one Spore from Colourless Strain.



D. *Sclerotial Formation.*

It will be evident from text-fig. 1 that the sclerotium consists of a mass of more or less firm tissue, enclosed in a thin layer of cells, in the walls of which the black pigment is located. Now, unless the sclerotium arises in a single hyphal cell, in which the variation may have occurred, the physiological change resulting in loss of colour must have taken place simultaneously in all those peripheral cells which normally would form pigment. In view of the fact that the new character is strictly hereditary, and obtains by the growth of any portion of a single individual, and that the initial colourless sclerotium arose in a tube culture containing a perfectly normal growth, such a deep-seated physiological change occurring simultaneously in a very great number of cells, and to an identical degree in all, is a process extremely difficult to conceive.

Very little is known of the initial developmental stages in the formation of sclerotia, but the latter are usually considered to be a further growth or modification of haptera, structures which are very accurately delineated in the works of MARSHALL WARD (80) and ISTVANFFI (33). In their formation one, or more usually several related hyphæ form short branches on which arise tufts of secondary branches. These are directed vertically to the contact surface, and the clustered hyphæ mat together laterally forming a solid body of a tassel-like shape and structure.

It is of interest to note that DE BARY (5, 6) regarded sclerotia and haptera as discrete bodies, stating that although clusters of haptera "have been mistaken for sclerotia . . . they have no connection." MUNN (56), who has most recently investigated this fungus, writes: "It was observed that in many cases the appressoria are incipient stages of the sclerotia (Plate 3, figs. 23, 24)." Now if MUNN's figs. 23, 24, and 13, which represent stages in the development of sclerotia, be compared with his figs. 25 and 27, which show the development of haptera, the essential difference is plain; and this distinction is obvious in the many delineations of these structures by previous investigators. There are no transition stages. The general laboratory study of the fungus and the relation of hapteral and sclerotial formation to various media and known conditions show that the structures are organs *sui generis*. This was confirmed in a very striking way by the development of these bodies in the aberrant strain. The haptera of the normal strain very rapidly become dark coloured and finally almost black, and this similarity of colouring with the sclerotia is in large part responsible for the confusion. In the case of the colourless strain, however, it is most helpful, for whilst the sclerotia have completely lost their pigment, the haptera have retained it, and thus in the one culture there co-exist the albinistic sclerotia and brown or olive-black haptera. This colour difference is distinctive from the inception of the two structures.

If therefore the sclerotia and haptera are discrete entities, the earliest stages that we know in the development of the former are the clusters of interweaving hyphæ

depicted by ISTVANFFI, MUNN, and others, and there is no reason why these should not have arisen in the branching of a single mother-cell. On searching selected cultures both by sectioning, and more successfully by mechanical teasing and *intra vitam* staining with neutral-red and methylene-blue, the initial stages were soon found. For the present issue, the main point of interest is that each sclerotium arises symphogenetically from an individual cell of the mycelium.*

On the open surface of a soft matrix the sclerotia usually remain separate, but if developed in a fissure or along a line of contact they often fuse to form a continuous crust. Figs. 50 and 51 on Plate 15 of ISTVANFFI'S memoir (33) are to be interpreted in this latter way. If mixed inocula of parental and colourless strains be grown, interesting composite sclerotial crusts may very rarely be obtained. In these there is no intimate mingling of hyphæ, but merely a lateral "grafting," and if such a composite sclerotium be germinated, the conidia and conidiophores, although indistinguishable, each reproduce purely their original parental type.

From the foregoing discussion it will be clear that the physiological change which resulted in the production of the aberrant form must have occurred in the mother-cell which gave rise to the initial colourless sclerotium in tube (E. 6).

6. NATURE OF LOSS OF COLOUR.

A detailed biochemical study of sclerotial pigmentation is beyond the scope of the present paper, but one may perhaps refer to certain simple experiments which throw some light upon the nature of the physiological change which has occasioned the production of the colourless form.

During the last two decades the pigmentation processes in animals and plants have been the subject of much investigation, and although this has followed the two distinct lines of biochemistry and genetics, the results have complemented each other in a quite remarkable way. The general trend of evidence would appear to lead to the view that the pigmentation processes throughout are essentially of an oxidatory nature with associated subsidiary or perhaps preliminary reduction processes.

Albinos have been shown to be divisible into two categories termed "dominant whites" and "recessive whites" respectively. In the latter, the absence of pigment is due to the lack of either the oxidase factor, or the chromogen factor, or of both; whilst in the former, both oxidase and chromogen are present, but their interaction is prevented by a third inhibiting factor. The oxidatory nature of the pigmentation processes is common to so many widely diverse organisms, that one might with some justification expect to find it even in the fungi, and consequently to find also that albinism in such an organism as *Botrytis cinerea* showed common features with the same phenomenon in rabbits or primulas. This fungus, however, is asexual, and the terms "dominant" and "recessive" are inapplicable; but if the pigmentation

* In certain strains of *Botrytis cinerea* the sclerotia originate meristogenetically.

processes are parallel to those of other organisms, the physiological changes which have resulted in the production of the colourless form may involve either the presence of a new inhibiting factor or the loss of either oxidase or chromogen or both.

The simplest and most direct method of examining the condition of the aberrant form is to test the colourless sclerotia for the presence of oxidase. If this be present and active it follows that the chromogen factor must be absent for otherwise pigmentation would have ensued. If there is no oxidase reaction, then either this factor is lacking or its activity inhibited by a third factor. The technique was based on that devised by KEEBLE and ARMSTRONG (41, 42, 43) in their investigations of flower pigments. Solutions of benzidine, α-naphthol, and p-phenylenediamine gave a marked reaction, whilst with a solution of guaiacum-resin a barely perceptible lavender-blue colour was obtained.

These reactions were unaltered by preliminary treatment of the sclerotia with absolute alcohol or hydrocyanic acid, or by the subsequent addition of hydrogen-peroxide, and in all cases they were produced in a few minutes irrespective of temperature differences ranging from that of the laboratory to incubation at 37° C.

The distribution of the oxidase in the colourless sclerotia was identical in every case, and was noteworthy, being confined to the walls of the peripheral cells which in the parental form contain the normal black pigment. So exact was this agreement that some of the sections of colourless sclerotia treated with benzidine were barely distinguishable from sections of normal sclerotia.

Whether the colourless sclerotia have been formed in light or darkness makes no difference to the intensity of the oxidase reaction, nor is this changed by growth of the fungus at various temperatures or upon nutrient substrata presenting very different physiological conditions.

On WHELDALE'S view (85) the chromogens are flavones or flavonal glucosides. The presence of these bodies in tissues may readily be detected by the bright colour given on exposure to ammonia vapour, and the dull-green (or sometimes red-brown) colour with ferric-chloride. When these tests were applied to sections and pulps of the colourless sclerotia a totally negative result was obtained.

NEILSON JONES (39) has shown that even in certain recessive whites both chromogen and oxidase appear to be present, but that owing perhaps to their discrete distribution interaction is prevented. To test the possibility of this explanation of the albinism of the aberrant strain a number of sclerotia were boiled in 5 c.c. of 50 per cent. alcohol to destroy the oxidase, pulped, filtered and the filtrate evaporated to a few drops. Sections of the colourless sclerotia were then immersed in this and incubated at 27° C. No trace of oxidase reaction was observed, confirming the absence of chromogen in the sclerotia.

True brown and black pigments are of the very greatest rarity in the higher plants. MÖBIUS (52) however has shown that they do exist as in the spots on the alæ of Vicia Faba, but even here the colouring matter is dissolved in the cell-sap.

In thin sections of the normal sclerotia of *Botrytis cinerea* the pigment appears as a true brown-black. Furthermore, as shown in text-fig. 1, it is contained in the substance of the cell-walls and not in the cell-content which is perfectly hyaline. It thus differs very markedly from the usual plant pigments, and would appear to be more closely related to the melanotic pigments, certain of the melano-proteins existing dissolved in the keratin structures.

The mother substance of melanin is generally supposed to be tyrosin, this colourless chromogen being converted into a black pigment by the oxidising enzyme tyrosinase. If, therefore, the black pigment in the normal sclerotia be melanin, the aberrant sclerotia which presumably lack the factor for tyrosin should exhibit a tyrosinase reaction. The tests for oxidase already referred to are only for the detection of phenolase or laccase which BOURQUELOT and BERTRAND (14), ZELLNER (88), PRINGSHEIM (63), KASTLE (40) and others have shown to be almost universally present in fungi.

Tyrosinase is comparatively rare in its distribution in fungi. BERTRAND (11), however, has shown its presence in species of *Russula*, especially *Russula nigricans*, and LUTZ (49) in the stipes and pilei of *Gyromitra gigas* and *Disciotis parlata* ; while more recently it has been demonstrated in *Lenzites sæpiaria* by ZELLER (87) and in various species of *Actinomyces* by DRECHSLER (24).

According to BOURQUELOT and BERTRAND (14) the best substrate for tyrosinase is tyrosin. Colourless sclerotia incubated at 18° C., 25° C., and 37° C. for 14 days in a saturated aqueous solution of silk-tyrosin gave a totally negative reaction, nor could this be changed by preliminary treatment with alcohol, ferrous sulphate or hydrocyanic acid or by subsequent treatment with hydrogen peroxide. A pulp of sclerotia, or sections, gave a similar result with this treatment.

CHODAT and STAUB (20) have advocated the use of *p*-cresol with the addition of glycin for the detection of the enzyme tyrosinase. Sclerotia immersed in this and incubated at 37° C. showed after many days a diffuse pale-yellow tint instead of the rich violet-blue reaction characteristic of tyrosinase.

In 1907 ABDERHALDEN and GUGGENHEIM (1) showed that N/100 hydrochloric acid permanently destroyed the activity of tyrosinase, but in the present case an immediate and direct oxidase reaction with benzidine was obtained after preliminary treatment of the colourless sclerotium with N/40 hydrochloric acid, although a strength of N/30 destroyed its activity.

It was found by BERTRAND (11) that tyrosinase could be differentiated from other oxidising enzymes by its lower lethal temperature, its activity being inhibited at 50° C. The oxidase in the colourless sclerotia is inhibited only by a temperature of from 80° C. to 85° C.

Furthermore BACH (3, 4) has shown that the pigment formed by the action of tyrosinase upon tyrosin may be oxidised further to a colourless compound by a dilute acid solution of potassium permanganate. The normal black sclerotia of *Botrytis*

cinerea remained unaltered when treated in this way. The black pigment is indeed extraordinarily stable, remaining apparently unchanged after boiling in concentrated acids and alkalies. When treated thus the stuff of the tissues disappears whilst the pigment remains as a most delicate gossamer-like cellular film. This stability immediately distinguishes the sclerotial pigment of *Botrytis* from other black fungal pigments such as the Aspergillin of *Aspergillus niger* which is soluble in hot water, or the colouring matter of *Daldinia*, *Hypoxylon* and various other carbonaceous Ascomycetes, which is soluble in alcohol.

The foregoing simple experiments only show (*a*) that the colourless sclerotia possess a direct oxidase system of the laccase type in a region corresponding exactly with that in which the black pigment is present in the sclerotia of the parental strain; and (*b*) that flavones or chromogens are lacking in the colourless sclerotia, or if present, they are inactivated in some way not understood. The experiments do not prove that the pigment in the sclerotia of the parental strain is due to the oxidation of a chromogen or that the aberrant strain has arisen by the loss or inactivation of a factor for chromogen. When, however, the above results are considered along with those of GORTNER (32), KEEBLE and ARMSTRONG (41, 42, 43) and others, which cumulatively form such positive circumstantial evidence, it is somewhat difficult to resist drawing the deduction that the albino strain of *Botrytis cinerea* has indeed arisen from the black parental form by the loss or modification of a factor for chromogen.

7. SIGNIFICANCE OF COLOURLESS FORM.

A. *Discussion of Recorded Mutations.*

The essential facts in the preceding pages may be summarised as follows: The colourless form arose spontaneously without any evident relation to external conditions or stimuli, in a single sclerotium of a culture which on accepted criteria was a pure line. The new form is apparently differentiated from its parent in respect to a single character only, that of colour, and this albinism would appear to be associated with the absence of a factor for chromogen. The change has occurred once only, and has given rise to a form unknown in nature and perfectly constant under all conditions. It would seem possible to place only one interpretation on these facts, that of mutation, and accordingly when I was privileged to exhibit this form to the Linnean Society of London on April 3, 1919, I described it as "an albino mutant of *Botrytis cinerea*."

Previous records of mutation in the fungi fall into two groups. In the first are those changes in genetic constitution which appear to bear a direct and purposive relation to certain conditions which have operated during the development of the organism or that of the preceding generation. As an illustration may be instanced the permanent acquirement of the power to ferment lactose by an organism previously unable to form lactase, this change being brought about by the growth of

the organism or its progenitor upon a lactose medium. The second category of mutations includes those cases in which the genetic change is apparently quite fortuitous, and this group is divisible into two sub-groups. In the first of these the change is associated with certain conditions which interfere with the normal metabolic reactions of the organism. For example, by treating the organism with toxic substances, or by subjecting it to extremes of temperature or desiccation, various indeterminate morphological or physiological changes are induced, such as alterations in spore dimensions or coloration, or growth upon standardised media. In the second sub-group the changes occur spontaneously under, so far as may be judged, perfectly normal conditions of development, and this category would include the colourless form of *Botrytis cinerea*.

Of the purposive mutations the classical case perhaps is that described by Massee (51), in which *Tricothecium roseum* was changed into a virulent parasite by habituating it to a previously immune host. This research to which credence is still largely given was, even for the year 1905, so inexact in all critical details that it may be now be regarded as of merely historical interest. Results worthy of serious consideration are described in the researches of Salmon (66, 67) on the adaptive parasitism of *Erysiphe Graminis* by means of growth upon bridging hosts, or the parallel results obtained with various rust-fungi by Marshall Ward (81, 82), Freeman (28, 29), Johnson (38), Pole Evans (62), and others. In none of this work, however, were the experiments carried out with single-spore strains of the fungi, under the rigidly controlled conditions imperative in such work. Nor was it realised until the more recent researches of Mains (50), Brooks and Cooley (15), Lauritzen (46), and others, how vital are the effects of even minute changes in the light, temperature, age, or humidity relations of the host plants. The findings of Kidd and West (44) on the importance of physiological predetermination in the life of the plant may also have a very material bearing on questions of relative susceptibility or immunity of host plants to fungal attack, and indicate factors which will be extremely difficult to control. Apart, however, from such collateral evidence as this, the value of bridging species in changing the physiological characters of the fungus is not supported by the work of Biffen (12), and has been directly contravened by the researches of Reed (64), and of Stakman and his associates (71–74). After much intensive study of this subject, the latter conclude "... the writers have not been able to detect any mutation nor to induce perceptible evolutionary changes experimentally." With rare exceptions such conclusions are supported by the invariable experience of all the critical and accurate workers in more recent experimental mycology, and there would appear to be little doubt that when a pure line organism is examined by a careful investigator under rigidly controlled conditions, heritable alterations bearing a purposive relation to educative treatment are absent.

The recorded instances of non-purposive genetic changes in organisms induced by

exposure to unusual conditions are very few in number. In 1908 ARCICHOVSKIJ (2) described a form of *Aspergillus niger* with yellow-brown spores which arose in a culture of the black form growing in Raulin's fluid to which 0·0001 per cent. of zinc sulphate had been added. Four years later SCHIEMANN (69) obtained various mutants of *Aspergillus niger* by treatment of cultures with potassium bichromate, exposure to high temperatures, and so forth.

In the same year WATERMAN (83) caused *Aspergillus niger* to mutate by treatment with 2 per cent. galactose, rhamnose, or glucose, 1 per cent. boric acid, *p*-oxybenzoic acid, or dichloracrylic acid. A fungus which the author speaks of as *Penicillium glaucum* was also caused to throw mutants by treatment with any of the following substances :—*p*-oxybenzoic acid, salicylic acid, trichloracrylic acid, tetrachlor-propionamid, pentachlor-propionamid, and pyrocatechetic acid (pyrocatechuic ?).

In all the above cases the new forms described differed from the parental fungi in many morphological and physiological characters, the most obvious being that of the colour of the spores. Furthermore, the production of the mutant forms was not a constant and specific reaction to certain definite chemical stimuli, but was merely a fortuitous circumstance resulting from the generally unfavourable conditions to which the organisms had been subjected. These were of such diverse nature that one might expect that almost any substance deleterious to growth or interfering with the metabolic processes in any way would produce similar results. Also not every culture subjected to these conditions gave uniform results over the entire growth, but only occasional cultures, and in these cultures only sporadic individuals, conidiophores or spores. Moreover, as SCHIEMANN (69) points out, mutations were also produced when the fungi were developing under favourable conditions.

Such results as these would have a somewhat indifferent reception if obtained in a physical or chemical laboratory. Their anomalous nature is stated clearly in SCHIEMANN'S paper as follows : " Die konstanten Farbmutationen sind beide auf mit $K_2Cr_2O_7$ versetzten Nährböden aufgetreten. Von einer spezifischen Wirkung des $K_2Cr_2O_7$ zu sprechen, ist trotzdem nicht möglich. Schon der Umstand, dass der Farbumschlag unter über 100 Kulturen mit $K_2Cr_2O_7$ nur zweimal stattfand, hier wiederum nicht die ganze Decke betraf, sondern unter vielen hunderten von Köpfchen nur einzelne, spricht dagegen. Ferner hat die *Proteus*-Mutante dieselbe Richtung eingeschlagen ; diese aber ist aus Hitzekulturen, einmal mit, einmal ohne Zusatz von $K_2Cr_2O_7$ hervorgegangen. Endlich sind nach braun abändernde Kulturen des *Aspergillus niger* auch sonst gelegentlich beobachtet worden—wenn auch selten—ohne dass das Chromal zur Verwendung kam."

In a discussion of the causes of genetic variation, BATESON (7) has written : "The state of knowledge of this whole subject is . . . most unsatisfactory, chiefly for the reason that in none of the cases which are alleged to show a positive result have two observers been over the same ground, or as yet confirmed each other . . . I do not know a single case which has been established and confirmed in such a way that we

could with confidence expect to witness the alleged phenomena if we were to repeat the experiment."

In the work of ARCICHOVSKIJ (2), SCHIEMANN (69), and WATERMAN (83), mutant forms were produced so easily and with such a diversity of stimuli and simplicity of technique, that it seemed probable that a repetition of their experiments might produce the necessary confirmatory evidence. Accordingly single-spore cultures of two strains of *Aspergillus niger* and of two strains of *Penicillium Italicum* were prepared. (WATERMAN used "*Penicillium glaucum*," but as THOM (77) points out, this "name as used at present seems to be applied collectively to the common green forms, which under examination are quickly found to be not one but several species.") With these fungi, the experiments of SCHIEMANN (69) and ARCICHOVSKIJ (2), and certain of those of WATERMAN (83) (galactose, glucose, rhamnose, boric acid, and salicylic acid) were carefully repeated. Although modifications in the colour of the spores, general morphological facies, and physiological reactions were observed, these affected the whole growth operated upon and to an equal degree; but the reproductive bodies of the modified fungi, when returned to the original conditions, gave in every case the original result. These changes were phenotypic and not genotypic, and throughout the whole series of experiments not a single mutation occurred.

More recently, experiments have been carried out with another strain of *Aspergillus niger* isolated from the air, and with a strain of *Penicillium* (*roseum* ?) from the soil, both these having been in culture in the laboratory for several months and repeatedly subcultured, although not derived from single spores. The fungi were subjected to rather gross treatment with various dilutions of toxic substances, such as silver nitrate, copper sulphate, mercury bichloride, lead nitrate, potassium acid phosphate, sodium chloride, and so forth, and then platings were made from these tubes. The resulting growths were perfectly normal.

In all work on the induction of genotypic alterations in micro-organisms, it is extremely difficult, even when the most scrupulous attention is given to every detail, to be quite certain that one has under control each of the almost infinite number of factors which may influence the results. Mycologists are only now beginning to learn how extremely complex and difficult to isolate such factors may be, and the researches of, for example, DUGGAR (25) and his collaborators on the physiology of certain fungi grown in culture media, or those of COONS (21) on the factors involved in the growth and pycnidium production of *Plenodomus fuscomaculans*, indicate the complexities underlying apparently the most simple physiological phenomena. Not the least interesting of the many experimental studies throwing light on the bionomics of *Aspergillus niger* are those of JAVILLIER (34) and of STEINBERG (75), in which it is shown how even such an unsuspected factor as the different quantities of zinc present in the glass of flasks, test-tubes, and other apparatus may vitiate comparative cultural studies of this fungus.

If, in the work of ARCICHOVSKIJ (2), SCHIEMANN (69), and WATERMAN (83),

accidental contamination did not occur, one is almost forced to conclude that some accessory factor of an unrecognised nature must have been operative in their experiments; a factor absent in my own repetition of their work and absent from the studies of every other mycologist who has carried out critical experimental investigations on these fungi.

Furthermore, as has been pointed out, these investigators emphasise the fact that their mutations are not of the nature of specific reactions to definite chemical or physical stimuli, but only the results of a generalised interference with the normal course of life of the organism. Thus SCHIEMANN (69) says: " . . . so zeigt sich als ein Gemeinsames, dass in allen Fällen, wo derartige Farbänderungen auftraten, Störungen der normalen Lebens-verhältnisse vorlagen. Es ist deshalb der Schluss berechtigt, dass die Mutation in den beobachteten Fällen durch einen starken Reiz ausgelöst wurde." But the experience of other investigators demonstrates the extreme genetic constancy of species of these fungi even when placed for many generations under the most unfavourable conditions. Numerous workers have shown that the morphological facies and physiological activities of fungi may within certain limits be changed at will in the single generation, but in no case, with the above exceptions, have these phenotypic modifications had any effect whatever on the genotypic constitution of the organisms.

B. *Alternative Interpretations.*

If the recorded cases of mutation in the fungi need not be accepted—and for my own part I do not feel compelled to accept them—how then is one to regard the colourless strain of *Botrytis cinerea*, which has been described in the present paper? Its fortuitous origin as a single sclerotium in a perfectly normal single-spore strain, whose development since its commencement is known, has already been emphasised. Under no conditions whatever, so far as is known, may the black sclerotial pigment of the parent strain, or the albinism of its derivative, be modified, and although other characters may show plasticity, no instance of a permanent alteration has been observed. Does the phenotypic constancy of the parental strain under constant conditions necessarily imply genotypic purity?

In the preceding account the parental strain has been spoken of as a "pure line," and on all accepted criteria this description is merited, for not only is it a single spore strain of an asexual organism, but it has been reproduced for most of its generations either by spores from single conidiophores or from single germinated sclerotia, both of which take origin in a single cell. In his work on 'Evolution by means of Hybridisation,' LOTSY (48) rightly insists that: "*Certainty of purity is a* conditio sine qua non *to obtain proof of the existence of mutation in living beings, just as chemical purity is a* conditio sine qua non *to obtain proof of the existence of mutability of the elements.*" Is there certainty of purity in the parental strain of *Botrytis cinerea* which gave rise to the colourless form? Further, is the fulfilment of such a

criterion possible in the fungi (or bacteria), and particularly in those forms for which mutation is claimed?

The only positive contribution to this subject is the work of BURGEFF (18), published in 1914, in which the results of the crossing of various forms of *Phycomyces nitens* are described. This fungus possesses a cœnocytic mycelium, containing numerous scattered nuclei. In the asexual form of reproduction spores are delimited within sporangial heads into which there have passed an indefinite number of nuclei. The multinucleate spores germinate and reproduce the cœnocytic mycelium. If, therefore, the original hyphæ are genetically impure, this condition will be maintained in all succeeding generations, for the sporangiospores merely reproduce the genetic condition of the hyphæ which give rise to the sporangia. Opportunity for genetic contamination occurs at sexual reproduction, for this process is merely a fusion of two multinucleate gametes to form a multinucleate zygospore, which on germination gives rise to a cœnocytic mycelium containing nuclei of both parental strains. There will be an equal chance for both types of nuclei to pass into the sporangia and be included in the multinucleate spores. A single-spore strain may thus be heterocaryotic. If now this form at sexual maturity fuses with a third form, and so on, the genotype of any particular isolation may be extremely complex. As, moreover, the sporangiospores are delimited and the walls of the zygogametes laid down without any apparent regard either to the condition, the number, or the position of the nuclei they separate, there is no absolute surety that two single-spore strains derived from an original single-spore strain will have the same genotype.

In such *Mucorineæ* it is, therefore, totally impossible to comply with the criterion of specific purity which is imperative in this issue. There is an almost equal lack of certainty of specific purity in the genera *Aspergillus* and *Penicillium*. The mycelium of these fungi is multicellular and each cell contains many nuclei. On asexual reproduction a vegetative cell gives rise to a conidiophore, and from this conidia are abstricted. The hyphal mother cell is multinucleate and these nuclei, by division and wandering, finally pass into the reproductive cells, these, in many species of the fungi, containing an indefinite number of nuclei. In other species the conidia generally contain but a single nucleus, but almost any preparation of such conidia shows multinucleate individuals.

A single-spore strain thus reproduces the genotype of its parent, and if the latter contains nuclei of different constitutions, this heterocaryotic condition will be maintained in the progeny.

Now, in the case of *Phycomyces*, genetic contamination occurs in the sexual process, for many of these forms are allogamous. There are, however, no species of *Aspergillus* or *Penicillium* known in which sexuality is necessarily allogamous, although there is no certainty that this type of reproduction may not occur, at all events, at intervals. We still know too little of what takes place in the sexual processes of either of these genera to make their discussion profitable; but given a

union of two parental mycelia only once in many thousands of generations, the mycelia of the offspring can afford no certainty of specific purity.

Moreover in any culture of a single-spore strain of either fungus it is not usually difficult to find two hyphæ which have come into contact, and the walls separating them have been auto-digested, giving cytoplasmic continuity. In a mixed growth of a number of strains these somatic fusions are also to be found, and although one is not able to state that fusions occur between hyphal cells of different strains, neither may the rare possibility of this be denied. If now from such a contaminated cell in *Aspergillus* or *Penicillium* a conidiophore were to arise, or either or both the sexual organs, the offspring of the reproductive bodies would be genetically impure, and asexual reproduction would only maintain this heterocaryotic or heterozygotic condition.

Thus even accepting the evidence of ARCICHOVSKIJ (2), SCHIEMANN (69), and WATERMAN (83), it by no means follows that the permanent changes they observed are necessarily mutations, for they may be interpreted in terms of genetic contamination, and in no case may these organisms comply with that criterion of "certainty of specific purity which is a *conditio sine qua non* to obtain proof of the existence of mutation." Although in this account very little reference has been made to bacterial mutation, there can be little doubt that the possibility of the rare occurrence of "somatic" fusions in these organisms places them out of consideration as subject organisms for the experimental induction of mutations.

Thus, according to the principle of parsimony, all so-called mutations in these groups of organisms may perhaps more wisely be interpreted in terms of the splitting of an originally impure genetic constitution or of gametic or somatic segregation from heterozygotes.

These considerations applied to the fungus *Botrytis cinerea* throw an entirely new light upon the value to be attached to the colourless strain. *Botrytis cinerea* possesses a multicellular mycelium, each cell of which contains many nuclei. The conidiophores are multinucleate and a small but indefinite number of nuclei pass into each conidium. Throughout the whole of the vegetative and reproductive mycelium the septa are laid down by a diaphragm-like growth from the hyphal walls, irrespective of the number or condition of the nuclei thus separated. Each conidium, therefore, merely reproduces the genetic constitution of the original cell of the mycelium in which its conidiophore arose. Many thousands of such asexual generations would, therefore, not alter the genetic constitution of the organism, and there is no sexual process. The possibility of genetic contamination is brought about by the occurrence of hyphal anastomoses. In the extremely rare chance of a fertile conidiophore arising from a cell contaminated by the nuclei or cytoplasm of a genotypically different individual lies, I believe, the explanation of the colourless form of *Botrytis* described. While this possibility exists, it is more consonant with the principles of scientific methodology to accept this interpretation than to formulate a mutational hypothesis of the origin of the aberrant strain.

Before concluding, a few words may perhaps be said concerning the *rôle* of the nucleus in the heredity of multinucleate fungi. The conidia of *Aspergillus*, *Penicillium* or *Botrytis* contain a variable number of nuclei, and yet if conidia are plated out the colonies which develop are essentially similar in physiological reactions and morphological properties for each particular species. It follows, therefore, that the exact number of nuclei which chance to be enclosed in each reproductive body has no bearing on the absence or presence of particular characters in the progeny, but is a purely fortuitous occurrence. What is vital, is, as BURGEFF (18) has shown, not the number of nuclei in each cell but the kind of nuclei present. Although the evidence on this point is meagre there would yet seem no reason to doubt that the structure of the nucleus in fungi is in general fundamentally similar to that in other organisms.

Following MORGAN (55) the chromosomes might thus be visualised as linear series of loci, like threaded beads. At each locus exist specific factors, making up a reaction system the elements of which bear a more or less specific relationship to one another. Thus, eight loci, representing the factor for black sclerotial pigment in the chromosomes of as many identical nuclei, would therefore have neither greater nor less visible expression than one locus in the chromosome of a single nucleus, or than 15 loci in the chromosomes of 15 nuclei in a single reproductive body. Increased number of nuclei does not signify a cumulative value for any particular character. But if owing to somatic fusion a hyphal cell containing eight nuclei with loci for black pigment became possessed of one or more contaminating nuclei from a genotypically different individual, and if this particular cell gave rise to a sclerotium, the characters of this sclerotium would be determined by the reacting systems of loci present in the two kinds of nuclei, the visible characters being the expression of the resultant of their interaction. The contaminating nuclei might find themselves in a cytoplasmic environment so foreign to their normal metabolism as to remain inert, or the locus for colour might be counterbalanced or cancelled by the locus for black in any one of the eight original nuclei, in which case the seven remaining nuclei would reproduce the original colour. In both cases the sclerotial characters would be wholly normal. On the other hand the loci in the invading nuclei might function as lethal factors, such as MORGAN (54) has shown to exist in the case of *Drosophila*, and development might be partially or entirely inhibited.

Again, the factors in the one system might react in many ways with those of the other system changing the physiological and morphological characters of the adult form so that apparently spontaneous variation in several independent directions might result, producing "mutants" such as those described by ARCICHOVSKIJ, SCHIEMANN and WATERMAN. The majority of the factors of the contaminating nuclei might remain inert, but one or more might react with certain factors in the original nuclei resulting in the inhibition of one or more of the latter. If, for example, the factor for chromogen were thus inhibited or destroyed the adult would be an albino similar to the new form of *Botrytis cinerea* described. If there are several factors for

colour, as would appear probable in the present instance, that associated with sclerotial pigmentation only might be inhibited, leaving unaltered the factors governing the pigmentation of spores or haptera.

On the other hand, it is not improbable that the contamination may be purely cytoplasmic, and the progeny of such a cell will therefore still be homocaryotic. The foreign cytoplasm, however, would create a new environment through which the nuclei would function, and this might so influence the development of the organisms as to alter materially the outward expression of one or more characters in the adult form. As the added cytoplasm would now be an integral portion of the organism, this changed expression would be permanent and heritable. It is thus possible for apparent "mutations" to occur in the fungi without any change in nuclear character. So far as the nucleus is concerned, regarding this structure as an entity distinct from the cytoplasm, the change would merely be phenotypic; but for the complete organism the change would be genotypic. It is perhaps in such purely cytoplasmic contaminations that apparent mutations in those organisms having uninucleate cells may be sought.

In the case under discussion, nuclear and cytoplasmic contamination must have occurred prior to the isolation of the single-spore strain, and yet the colourless strain arose only in the third tube-generation subsequent to this. If, however, one assumes a contaminated original strain, it is not inconceivable that in the fortuitous septation of the hyphæ, certain nuclei and cytoplasmic matter were separated, such that in all things save chromogen formation the resultant resembled the parental strain. The expression of this resultant would be an albino strain such as that described. Such a concomitance of conditions might well appear almost inconceivable, but the appearance of an albinistic strain of the fungus is equally almost inconceivable, for in the history of the genus as we know it it has occurred but once.

It would seem to me probable that the same concept which Bateson (8) and Lotsy (48) have formulated for the higher organisms may hold true for both fungi and bacteria, but here the recombination of genes may not only take place by sexual fusions, but also by direct "contamination" of one species by the nuclei and cytoplasm of another in vegetative anastomoses. New reacting systems of chromosomal loci impinge upon each other, and the resultant is the new form. This at first may be heterocaryotic, but if true sexual processes occur later, the offspring become homocaryotic but heterozygous. Such a hypothesis fits the facts and explains them as I think no other present hypothesis does.

It is not the possibility of mutation in the fungi (and bacteria) that is here denied, but rather the compulsion to accept that interpretation of the evidence as it now stands. As the facts present themselves to me, avenues of interpretation other than that of mutation are still widely open.

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DESCRIPTION OF PLATE.

Fig. 1.—Petri dish culture in which the normal form and its colourless derivative have been grown. Pigmented haptera of the albino strain may be seen at the periphery of the culture in contact with the glass. × ⅔.

Fig. 2.—Vertical section of colourless sclerotium. × 250.

Fig. 3.—Vertical section of parental sclerotium. × 250.

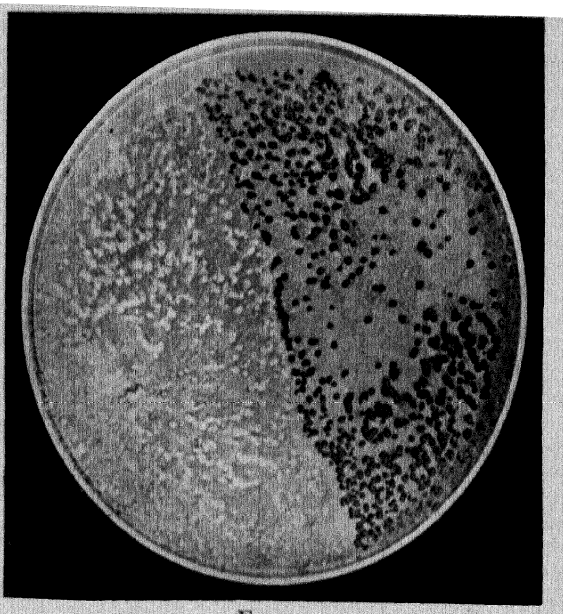


FIG. 1.

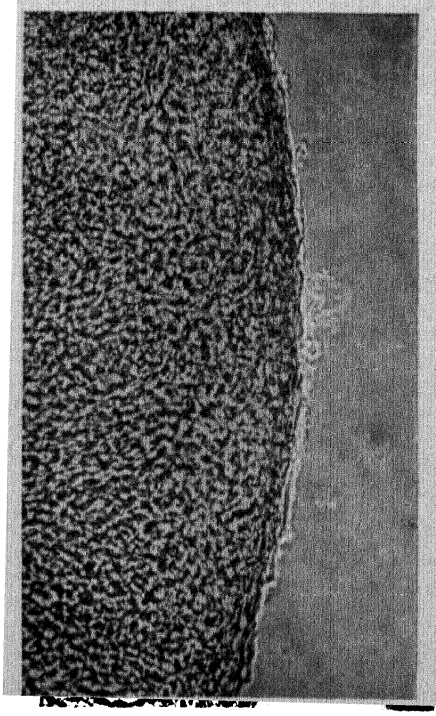


FIG. 2.

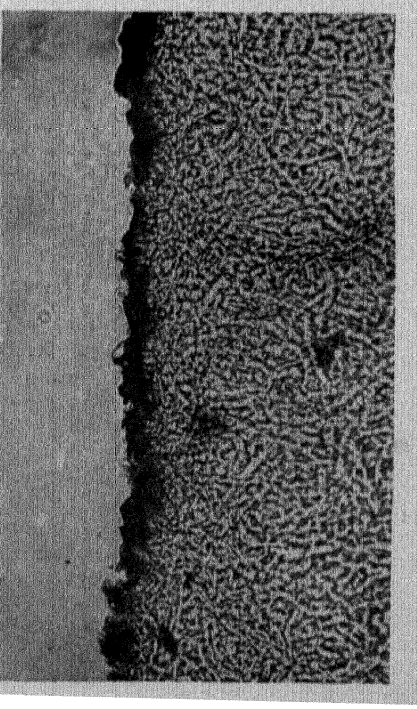


FIG. 3.

IV.—*The Irish Eskers.*

By J. W. Gregory, *D.Sc., F.R.S.*

(Received February 6, 1920,—Read April 29, 1920.)

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I. The Theories of Eskers.

The term esker is Irish and its definition should be settled by reference to the eskers of Ireland. They are hills of sand and gravel, which are typically ridges, but are sometimes mounds or groups of mounds. Excluding various suggestions only of historic interest, there are three chief theories of the formation of ridge-eskers. According to the first theory, due mainly to Hummel (1874, p. 3), eskers are accumulations of gravel along sub-glacial rivers. According to the second theory, due largely to Holst (1876–7, p. 97), eskers are deposited along rivers flowing either over, or perhaps partially within, glaciers. According to the third theory, which was advocated almost simultaneously by Hershey (1897, p. 241), Baron de Geer (1897, pp. 377–386), and Baron von Toll (1899, p. 22), eskers are the deltaic deposits of glacial rivers, and their ridged form is due to their continuous deposition at successive positions by the slow recession of the river mouth during the retreat

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of the ice sheet.* An esker so formed should show an annual banding due to the seasonal variations in the volume of the glacial river.† This banded structure is well developed in the Swedish osar (åsar), for many of which this receding delta theory may be regarded as established.

Prof. SOLLAS adopted HUMMEL'S theory for the Irish eskers. He described them (1896, p. 803) as the casts of systems of sub-glacial rivers. He justly objected to the supraglacial theory, since the eskers were clearly accumulated in their present positions (*ibid.*, p. 819) and had not been redeposited at a lower level on the melting of the ice. His map of the esker system of Central Ireland, compiled from the maps of the Geological Survey, shows some eskers arranged in convergent series like a river and its tributaries; and it was this arrangement which suggested the fluviatile origin of the osar of Sweden.‡ The Irish eskers have therefore been regarded as exactly equivalent to the Swedish osar.

The literature on the Irish eskers deals mainly with their distribution and general structure. It is therefore advisable, before considering their origin, to record some detailed evidence regarding their composition and intimate structure.§

II. IRISH ESKERS.

1. *The Eskers near Tullamore and Kilbeggan.*

One of the most important and instructive esker systems is that of Ballyduff and Newtownlow, to the north and north-east of Tullamore. It has been described by J. O'KELLY and illustrated by DU NOYER, in 'Mem. Geol. Surv., Ireland,' Explanation Sheets 98, 99, 108, 109, 1865, pp. 29–32. JUKES, in the introduction to that memoir (p. 8), refers to this group as including "the most remarkable eskers yet mapped in Ireland." The esker has the plan of a horseshoe, situated around the town of Clara. Its north-western arm appears to be an extension of the well known Streamstown Esker, and its southern arm to be a continuation of the long east-and-west trending esker through Seven Churches and Ballinasloe. The plan of this esker system, as shown on Prof. SOLLAS'S map (1896, Plate 69), appeared consistent with its formation by rivers which converged from the north-west, west, and south-west, and which continued eastward toward the Liffey through the two east and west

* VON TOLL clearly summarises his conclusions as follows:—"Die Bildung der Åsar am ehesten als das Product von Gletscherbächen anzusehen sind, die aus dem Thore eines Schritt für Schritt sich zurückziehenden Inlandeises hervorbrechen, oder mit anderen Worten als die Vereinigung einer Reihe auf einander folgender Schuttkegel" (1898, p. 22).

† I. C. RUSSELL (1893, p. 241) also adopted the view that the banding of eskers, or osar as he called them, was due to periodic variations in the streams.

‡ This arrangement is shown, *e.g.*, on the map by HUMMEL, 1874, Plate II, or in the figure by J. GEIKIE, 1894, p. 481.

§ The most detailed description is that in 'Mem. Geol. Surv.,' Sheet 112, of the Greenhills Esker, near Dublin, which is, however, especially in the part most fully described, not a typical example.

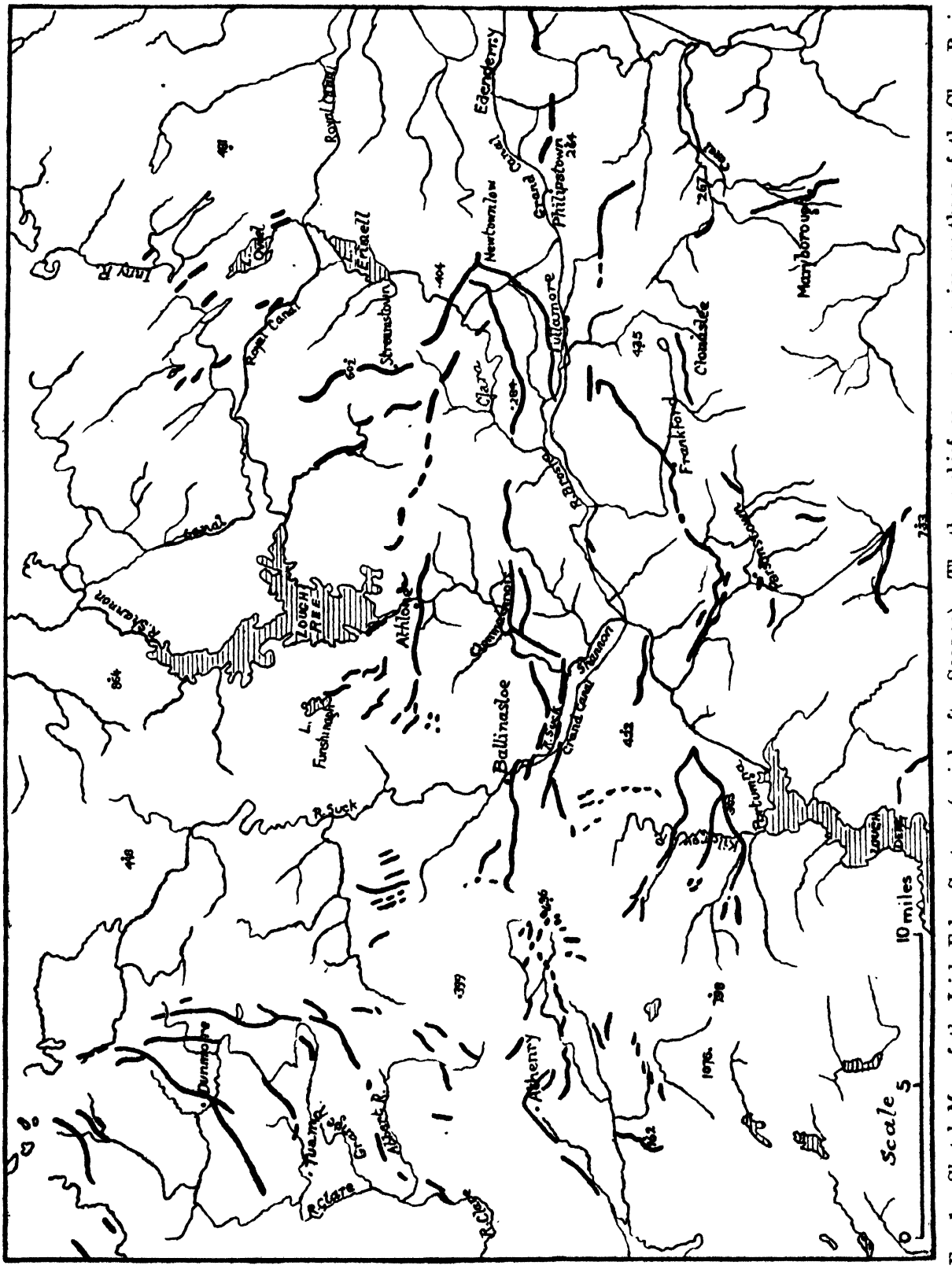


FIG. 1.—Sketch Map of the Irish Esker Systems (mainly after SOLLAS). The three chief convergent series are those of the Clare Basin, near Dunmore, of the Kilcrow River, near Lough Derg, and of the three esker lines which converge towards Newtownlow.

eskers to the south and south-west of Edenderry. From them it is separated by a gap of about eight miles, which might be due to subsequent denudation.

The Ballyduff Esker is exposed in gravel pits beside the roads going north-west and north from Tullamore. The esker begins about 5½ miles west-north-west of Tullamore, and good sections of it are exposed two miles north-west of the town by Ballyduff Bridge (fig. 2). The core of the esker there is a ridge of well bedded sands and fine gravel with a slight dip to the north. Four layers of clay are interbedded in the sands. These beds have been cut through by two faults with a downthrow to the north. These faults were contemporary with the formation of the esker, because the coarse gravel which forms the upper sheet of it passes unbroken across these faults. This fact is fatal, for this esker, to the supraglacial river theory. On the southern side the bedded sands have been denuded, and a coarse torrential gravel occupies the hollow thus formed, and passes upward into a fine-grained bedded gravel, which on each side dips away from the crest of the esker. The sands of the esker core were obviously once much wider, and are the remains of material deposited in quiet water. The faulting was probably produced by subsidences consequent on the melting of masses of ice included in the sands, as after the faults were formed powerful currents of water spread a sheet of coarse gravel over the ridge of sand.

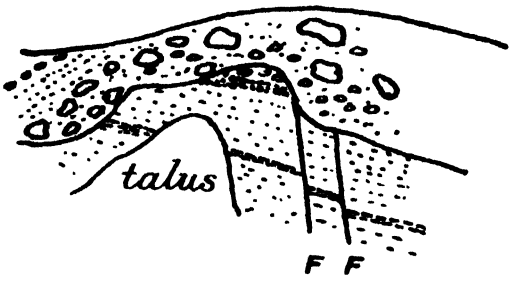


FIG. 2.—Section in an Esker, with contemporary faults. Ballyduff Bridge.

Sections on the southern slope of the esker beside the road to Kilbeggan, 1½ miles north of Tullamore, expose at the base a coarse bouldery wash, which passes laterally to bedded sand and loam containing irregular masses of gravel and steeply inclined shoots of clay. The clay has a very steep dip and appears to have been dumped in its present position by slipping down a steep bank. The upper part of the esker consists of a regularly bedded loam.

The arched structure, which is often described as characteristic of eskers, is absent from this pit and from most of the sections in the Tullamore Eskers. Thus 2 miles north-east of this pit, to the south-east of Derrygolan Bridge (fig. 3), the base of the esker is a bed of stratified gravel with a flat upper surface which has a very slight inclination to the north. This bed is covered by a layer crowded with limestone and chert boulders, which are as much as 15 inches in diameter; they are all fully water worn, and lie horizontally. Some of the layers in this bouldery wash consist of closely packed cobbles and pebbles, and contain but little sand. The bedding

planes are cut off abruptly by the slopes of the esker (fig. 3), which is therefore the remnant of a once wider ridge.



FIG. 3.—Section of an Esker, with horizontal bedding. Derrygolan Bridge.

Deeper sections in the same esker are exposed a mile further to the north-east at Murphy's Bridge The base of the esker there is a bed of sharp sand containing some well rounded grains. The upper part is a bed of stratified dark to greenish sand and gravel, which contains a few small boulders and dips regularly to the north. This bed and its bedding planes are abruptly cut off above by from three to five feet of coarse bouldery gravel, which forms the surface of the esker (fig. 4).

Near Newmill Bridge, a mile further north-east, are some excellent sections in the part of the esker sketched by DU NOYER (figs. 9 and 10, 'Mem. Geol. Surv., Ireland,' Explanation Sheet 98, etc., pp. 30–31). Three large adjacent pits show striking variations in the material. The south-western pit beside the old limekiln is characterised by its regular bedding. The base is a coarse wash with boulders a foot in diameter; above this is a layer of well bedded loam and clay, which is covered by a bed of sand containing large boulders. This is covered by a layer from 2 feet to 6 feet thick, which is crowded with boulders up to 2½ feet in diameter. At the top of the esker is a bed of gravel 10 feet thick, with inconspicuous bedding and boulders up to a foot in diameter. The planes between the successive beds are cut off abruptly by the southern slope of the esker, showing that the beds were once more extensive.

Immediately to the north of this pit, on the opposite face of the esker, is a pit in sand with very confused false bedding. It offers a striking contrast to the regular bedding and coarse boulders and gravel of the southern pit.

The third pit, the north-eastern, is a confused mass of bouldery wash. The only evidence of stratification is afforded by occasional horizontal patches of large boulders. Some of the boulders are standing on end. The material in this pit, owing to the size and arrangement of the boulders, is very morainic in aspect; it resembles the redeposited moraine stuff of some of the larger Swiss moraines.

A little further to the north-east the esker turns abruptly north-west and is continued as the Newtownlow Esker and Long Hill. Good sections are exposed at Newtownlow. The base of the esker consists of coarse gravel, including patches of bouldery wash. Above this gravel is a thick bed crowded with large boulders, and against its steep western margin the bedding planes in the gravel have been inverted. This disturbance was contemporary (fig. 5); for this boulder bed and the gravels beside it have been planed off and a layer of sand is continuous across the

junction. This sand is covered by bouldery wash which usually forms the surface of the esker.

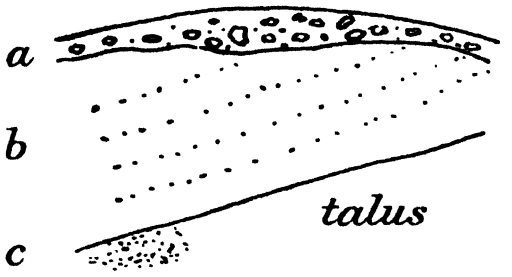


FIG. 4.

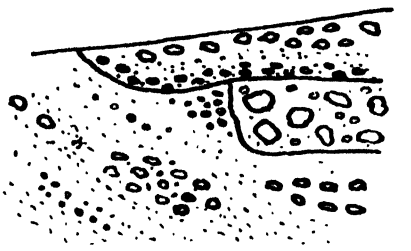


FIG. 5.

FIG. 4.—Section in an Esker at Murphy's Bridge, with fine sand near the core and coarse boulder drift on the top.

FIG. 5.—Section in an Esker, showing great contemporary erosion. Newtownlow.

Three miles west of Newtownlow, to the south and south-west of the village of Kilbeggan, is a long low esker parallel to the Long Hill Esker. It lies on the floor of the area included within the horse-shoe course of the Newtownlow, Ballyduff and Clara Eskers. Some shallow sections in the gravel pit near the fork of the road half-a-mile south of Kilbeggan show the general character of the material. The western end of the pit consists of beds of sharp sand separated by gravel, in which the pebbles and the few boulders present are all well rounded. The aspect of this material is like that of a river gravel. Eighty yards along the pit is a section of stratified sand and fine gravel passing up to gravel with some boulders. At the eastern end of the pit is the following section in descending order.

Soil: 5 feet of unsorted boulder drift; 2 inches of sand; 4 inches fine chert gravel; 18 inches stony loam, including 12-inch boulders; 1 inch to 10 inches irregular layer of sharp sand; 1 inch to 12 inches loam with very few pebbles; 2 feet stratified gravel, part of a lens which is 25 feet long and 3 feet at its thickest; 18 inches stratified sand with pebbles.

The general character of the Ballyduff-Newtownlow Esker is inconsistent with the glacial river theory. The extreme difference between adjacent parts of the esker, as shown at the three pits near Newmill Bridge, indicate that its material had been brought from the side and not carried along the esker. Its original formation can best be explained by the accumulation of material washed from the adjacent front of a melting ice sheet. The esker is not, however, a moraine, as its thoroughly water-worn character and bedding show that it was deposited by sheets of water. The material had obviously not been carried far by water, and its lateral variations are dependent on the nature of the drainage from the ice front. The material in the north-eastern pit at Newmill Bridge probably accumulated where the ice front was a steep wall, at the foot of which the boulders collected in heaps. The false bedded sand of the middle pit was deposited where a stream flowed from the ice. The wide-

spread horizontal sheets of alternate sand and gravel in the south-western pit were doubtless due to variable floods of water.

The composition and structure of the Ballyduff-Newtownlow Esker, therefore, indicate that it was a marginal formation produced by the wash of material from a receding ice front; and this explanation agrees also with the general plan of that esker system.

The distribution and levels of the Parsonstown, Clara and Streamstown Eskers show that they are marginal formations beside an ice sheet which came from the north-west down the Suck and Shannon Valleys.

The Kilbeggan Esker, on the other hand, is like the Swedish osar, fluviatile in character and was probably deposited as a delta formation at the receding mouth of a glacial river.

2. *The Athlone Esker.*

South of the railway to the west of Athlone a large pit exposes a high section of a very coarse stratified gravel containing in one part closely crowded boulders up to 2½ feet in diameter. Owing to the size and number of these boulders the material looks more morainic than fluvio-glacial. All the material has, however, been water washed, and the boulders were probably rolled from the ice down a steep bank of pebbles, so that they were easily moved a little distance from the actual edge of the ice.

This morainic type of esker continues west of Athlone and is well exposed near Mount William, about 1½ miles west of Athlone, in the large ballast pit north of the railway. The pit illustrates the variable structure of this esker. The south-eastern part is strewn with big boulders, up to 6 feet in diameter; some of them show glacial striæ of the curved, blunt, short type, like those formed by the twisting movement when stranded ice is swung by the tide. The south-western corner of the pit is in a thick deposit of well bedded sands, which are in places false-bedded and contain some pebbly layers. At the north-western part is a projecting mass of coarse-grained angular faceted gravel, which has been cemented to a hard conglomerate, while the interbedded soft material has been worn into caves. The eastern part of the northern face of the pit exposes a variable series of boulder drifts, gravels, sands, and loam; the upper layer is usually a boulder drift overlying sand, with puckered and faulted seams of clay. In part of this face the beds have been violently disturbed. Towards the eastern end the beds have a dip of 25°, and there are three definite boulder beds inter-stratified with coarse bedded sands.

The beds in this pit include morainic drift along the southern side, sheets of sand which had been deposited in comparatively quiet water, and alternate beds of boulder bearing gravel and sand.

The Athlone Esker is of the marginal morainic type; but the smaller eskers which trend north-westward from it have a well developed banding. Thus about a mile to the west of the Mount William pit in a small pit a little east of Millbrook, the upper-

most bed is a 3 foot layer of coarse drift containing boulders up to 18 inches in diameter; below is a layer of coarse sand, and then another 3 feet of bouldery drift containing boulders 20 inches by 30 inches. The stones in both gravels are water worn, and many of the pebbles are as rounded as in a river gravel. I saw in this pit no trace of glacial striæ. The banding shows variations in the rate of deposition, but there is no evidence to show whether it was due to seasonal changes.

Two miles further to the west-north-west, beside the junction of two roads, just south-east of Ballymullakill, is a large gravel pit, the face of which is partly overgrown, and the relations of the beds in places obscured by slipping. The pit, however, gives evidence of frequent alternate increase and decrease in the currents by which these deposits were laid down. In the length of 85 yards occur seven bands each composed of small boulders, cobbles, and coarse gravel; these bands are separated by layers of sand with small cobbles and pebbles. These bands dip to the north. At the southern end of the pit is a bed of gravel with a steep eastward dip; it has been cut across by a channel filled with boulder drift.

The banded structure of the esker is also shown a little further along it in a pit to the north of the road, half-a-mile west of Ballymullakill and a mile and a quarter south-east of Brideswell. Parts of this pit are overgrown; but the section shows, in a length of 100 yards, eight beds of bouldery drift which are separated by beds of sand and fine gravel (figs. 6 and 7). The beds dip to the north.



FIG. 6.—Longitudinal Section in the osar at Ballymullakill, north west of Athlone, showing the repeated beds of coarse bouldery gravel. Heights in feet; lengths in yards.

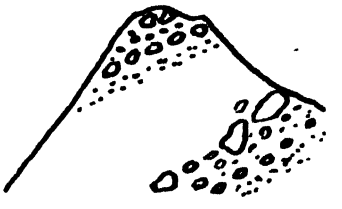


FIG. 7.—Section in the osar north-west of Athlone.

These and other pits between Athlone and Lough Funshinagh give clear evidence that this esker was formed by a stream flowing south-eastward, and that the banded structure was due to the varying strength of the current. It is tempting to regard the variation as seasonal, the succession of coarse to fine material representing the variation during one summer's melting. This banded structure is, however, so irregularly developed that it would be unsafe to conclude that the successive bands represent deposition in succeeding years.

The character of this banding could best be determined in the winter, when the coarse bands of material could be recognised beneath the turf. Detailed mapping of some of these longitudinal eskers would probably determine whether their banding is a seasonal stratification.

The Athlone Esker breaks up westward into a series of irregular mounds and short ridges, which are well developed around Castlesampson. Eastward it passes Moate, and probably continues as the ridge thence northward and north-westward along the valley of the Tang River, a tributary of the Inny, to the south-east of Ballymahon.

The Athlone Esker appears to have been formed by a glacier which came down the Shannon valley and through Lough Ree. It was bounded on both sides by driftless highland—to the west by that, over 500 feet high, south of Roscommon and east of the Suck valley; to the east by that south-east of Longford and that, over 400 feet high, east of Lough Sunderlin and Ballymore.

3. *The Ballinasloe-Clonmachnois-Clara-Streamstown Esker.*

To the south of the Athlone Esker is one of the longest, most regular, and best developed eskers in Ireland. It begins about eight miles west of Ballinasloe; its general course is from west to east, though it makes successive curves southward through Ballinasloe, New Town, and Cornaveagh. It is there breached by the Shannon, on the eastern bank of which, at Creevagh, it is joined by a branch which passes south of Ballinasloe through Glenlougham, Kellysgrove, and Shannon Bridge. The combined esker runs from Creevagh to Clonmachnois, where it again subdivides; the northern branch is the Pilgrims' Road Esker, and the southern branch continues due east through Clonfinlough and Togher, and, after a gap made by the valley of the Brosna, to Clara. It passes Durrow, south of Kilbeggan, unites with the Ballyduff-Newtownlow Esker, and bends sharply back and forms the narrow ridge known as the Long Hill; it crosses the Brosna valley, continues further north as the well known Streamstown Esker, and ends in the esker near Moyvore.

This esker is about 65 miles in length from its western end near Woodlawn, on the watershed between the Shannon and the Dooyertha (a stream entering Galway Bay), to its north-eastern end in the Inny valley; it is not, however, continuous for the whole distance. The esker receives a series of branches, and affords one of the best Irish examples of an esker with confluent tributaries, such as suggested the formation of eskers by rivers in glacial canyons. If that had been its origin, the combined river would have been formed by three main streams. The north-eastern stream would have deposited the Long Hill of Newtownlow, and would have been joined by tributaries from Streamstown, Ballymahon, and Athlone. The second main stream would have come from Ballinasloe, Clonmachnois, and Clara; and its chief tributary would have been that of the Shannon Bridge Esker. The most southern of the three main streams would have come from Tullamore and have carried the drainage from

Parsonstown, Eyrecourt, and Portumna. The outlet of the three combined rivers would have been past the fragmentary eskers south of Edenderry.

I have examined this esker at a good many localities and failed to find that its structure agrees with the glacial river hypothesis. The shape of the boulders is not that typical of river gravels. In most places glaciated stones can be found, whereas the striæ disappear after transport by water for a very short distance. Even when no striæ are observable the boulders and pebbles commonly have flattened surfaces, characteristic of those of boulder clay. In some bands of gravel the stones have all been washed and rounded; but these bands form a small proportion of the constituents of the esker, and the abrasion of their stones and removal of the striæ may have been by surf action or by small local streams. The deposits are often unbedded or show only very faint and obscure traces of bedding. In some pits the material appears to have been merely dumped where it lies. The boulders may be packed very closely and large boulders often stand upon their ends. The aspect of many pits is morainic, and they include layers of sand or clay deposited on the floors of depressions in the drifts.

This esker and its branches appear to have been formed on the margin of an ice sheet, which came from the high grounds to the north-west in Mayo and eastern Galway, and deposited ridges of drift at various stages in its retreat. The branching of the esker appears to be due to the ice margin having remained stationary at one place, while it receded in a neighbouring locality. Thus the glacier held its position near Newtownlow while the ice front in the Shannon valley receded about 15 miles.

The evidence for these conclusions may be summarised as follows:—

The Ballinasloe Eskers.—West of Ballinasloe the esker is well exposed in pits beside the railway near Kilmalaw Bridge, north of Aughrim. The esker there consists of a confused mass of closely packed boulders, which are especially numerous in the top layer. Rough bedding can be seen in some layers. The largest boulder observed in the pit at Kilmalaw Bridge was 2½ feet long. The boulders are often ice-scratched. Half a mile to the east of the Kilmalaw pit is another gravel pit of the same type. The bedding is obscure and the material appears to have been dumped. The boulders are irregularly packed.

Between 1½ and 3 miles west of Ballinasloe, to the north of the railway line, is the Kilcloony branch of this esker. It is well stratified and consists largely of alternations of boulder drift and gravel, with some layers of sand. A pit south of the road about halfway along this esker shows a 6-foot bed of closely packed boulders, some of which are vertical. Beneath the bouldery drift is a bedded, fine-grained gravel. The structure of the eastern end of the Kilcloony Esker is well shown in two pits to the west of the lane leading to the church. The southern pit includes two beds of bouldery drift, separated by 10 feet of gravel. The beds have a steep dip to the north. In the northern pit the gravels are covered by 3 feet of false-bedded sand with lenticles of well-washed gravel; but in a layer of coarse gravel immediately

beneath the sand are many well ice-scratched stones. The gravels include pebbles of a fine-grained gray biotite-granite and foliated granites which doubtless came from western Galway. The chief deposits of sand in these pits occur, as usual, on the northern side of the esker, as if they had collected in water between the esker and the receding ice front.

Half a mile south-east of Ballinasloe, on the western side of the canal just north-west of Somers Villa, is a gravel pit in the main Ballinasloe Esker. The core of the esker consists of a coarse bouldery drift with some interstratified beds of subangular gravel. It is capped by bedded sand and fine angular gravel which, on both sides of the esker, have been faulted. After the formation of these faults a layer of coarse pebbly drift was deposited across the esker, so that the faulting was clearly contemporaneous with its deposition. A bed of sand occurs on the northern slope of the esker. Some of the boulders are striated, and after the striation were encrusted by calcareous layers, one of which Madame LEMOINE has identified as a Lithothamnium. This esker rests on boulder clay, which is exposed in a pit beside the towing path of the canal, and the upper part of this boulder clay contains boulders bearing marine encrustations showing algal structure.

The Shannon Bridge branch of the Ballinasloe Esker is well shown in a pit $2\frac{1}{2}$ miles south-south-west of Ballinasloe, near Kellysgrove, and in other pits near Sinclair's Village and by Glenlougham Church. A section across this esker at Kellysgrove was figured in the 'Geological Survey Memoir' (Sheets 96, 97, etc., 1867, p. 25), and shows that the stratification is not in the same direction as the outer slopes. The bedding is arranged as a trough and not as an arch.

A pit just north-west of Sinclair's Village shows the close packing of the boulders which sometimes occurs in this esker. The boulders lie at all angles; they are not striated. Their matrix consists of narrow partings of a coarse sand, which includes some cobbles and small boulders. The largest boulder in the area sketched was 4 feet long by $1\frac{1}{2}$ feet thick, and a 6-foot boulder lay on the floor of the pit. A few of the pebbles are of porphyritic granite. The aspect of this pit (see fig. 8) is that of a moraine with closely crowded boulders.



FIG. 8.—Part of Face in a Pit N.W. of Sinclair's Village, near Ballinasloe, showing crowded boulders. Scale, 1 inch = 5 feet Boulder *a* is 4 feet × $1\frac{1}{2}$ feet; boulder *b* is 3 feet 8 ins. × 1 foot 10 ins.

Kellysgrove Esker.—At Glenlougham, to the west of Kellysgrove, the core of the esker consists of false-bedded gravel, with boulders and patches of sand. The sand

patches consist of irregularly deposited lenticles. The upper part of the pit is in roughly bedded bouldery drift of morainic aspect. On the south side this bouldery drift is intercalated with wedges of gravel and coarse cobbly gravel. A short search yielded in the gravel six specimens of Galway granite.

Shannon Bridge Esker.—The Kellysgrove Esker is a continuation of the Shannon Bridge Esker, the structure of which is well exposed in pits south of the Ballinasloe road, west of Shannon Bridge. A long, shallow section on the northern face of the esker shows a crowded medley of boulders which are up to 2½ feet in length; this bed overlies a black chert sand, with a steep dip to the north. The esker contains a few thin layers of clay. The deeper pits on the southern side show an unwashed, unsorted material, which is arranged as a confused dump. The structure of the esker agrees with its origin as a morainic deposit.

East of Shannon Bridge the esker bends to the north-east, and follows the eastern bank of the Shannon until it joins the Ballinasloe Esker near Clonmachnois. At Cloneff, south of the junction with the Ballinasloe Esker, the Shannon Bridge Esker consists mainly of sharp, angular, bedded sand, with lines of water-washed pebbles. This material would pass as fluvioglacial, but the sections are small and obscure. The nature of the esker must be judged by its characters where better exposed.

A little further north, to the west of Lough Nanag, the esker branches, and encloses a series of pits and hollows shown in the detailed map and sketches published in the 'Geological Survey Memoir' (Sheet 98, etc., pp. 24, 25, 26). A pit to the west of the road shows coarse bouldery drift overlying a bed of ill-sorted water-worn gravel; the boulders lie at all angles; some are vertical, and occur in a coarse pebbly sand.

Clonmachnois.—Just east of the Seven Churches at Clonmachnois, three-sixteenths of a mile east of the school, some shallow excavations show an irregularly dumped cobble gravel with faceted boulders, on which I did not see any striæ, and irregular patches of loam. There is no sign of deposition by streams of water. The material appears to have been dropped irregularly in water.

From Clonmachnois, two branches of the esker continue eastward; the northern branch is that of the Pilgrims' Road. The southern branch goes easterly, and extends, though with one gap of nearly four miles, to Clara, around which are many large and instructive sections.

Clara.—North of Clara Church are some deep pits in drift resting against a plateau. The lower part of the drift consists of false-bedded sand and gravel, with a prevalent dip to the east. The upper part is a bed of bouldery drift, 20 feet in thickness, which contains irregular patches of sand and fine gravel. The upper bed has in places been cemented to a conglomerate, which overhangs the underlying gravel. In the eastern part of this line of pits the gravels are covered by laminated false-bedded sand and pebbly gravels, with occasional boulders; these beds are in places faulted, and include large pockets of bouldery drift.

The material in these pits is described in the 'Memoir of the Irish Geological Survey' (Sheet 98, etc., pp. 28 and 29) as an esker; but it is admittedly not an isolated ridge, for the beds flank a Carboniferous Limestone plateau, part of which is driftless. This material was apparently deposited where an ice sheet from the north-west melted against the side of the plateau. Some of the material was washed and sorted, and patches of sand collected in the depressions. The beds have been faulted and disturbed by slips during the settlement of the material.

East of Clara the Clonmachnois Esker subdivides. The southern branch is followed by the Tullamore road for a couple of miles, and then bends northward, reuniting with the northern branch near Durrow Abbey. The northern branch crosses the Brosna River, and then turns south-south-east, forming a ridge, which occasions the remarkable meander of the Brosna about a mile and a half east of Clara. Between the two branches of the esker the land is level and low.

The structure of the two branches of the Clara Esker may be illustrated by reference to the sections at the pit at Lehinch, and by the large gravel pits at Ashfield, $1\frac{7}{8}$ miles from Clara, on the road to Tullamore. The Lehinch pit is of interest, on account of the occurrence of a band crowded with fragments of minute calcareous tubes, some of which grow in tufts. The pit is on the eastern side of the road leading to the bridge over the Brosna, at the end of its horseshoe loop. The core of the esker at this point consists of sand and fine gravel, which are arranged in a shallow trough. At the west-south-west end the sands and gravel dip 50° to the south-west. This material is abruptly cut off above, and is covered by a bed of gravel composed of rounded and angular cobbles. Similar material covers the middle of the esker, and, on the other side, slopes down to the east-north-east. It is there covered by a bed of laminated sand and fine gravel, which dips 20° to the east-north-east. This material has the characteristics of an angular beach gravel. It is 10 feet thick. Below it is a layer, 1 foot in thickness, of coarse, loosely packed boulders, the largest of which are about 10 inches in diameter. In the sand between these boulders are many joints, and occasional tufts of calcareous tubuli. Many of the stones in this layer are encrusted by calcareous tubuli. Beneath this bed is a 9-inch layer of fine-grained gravel, also containing encrusted stones. Below are 18 inches of an angular beach gravel; it rests on a 6-inch boulder bed, in which the boulders are encrusted by tubes.

The Ashfield pit, on the southern branch of the Clara Esker, is better known, as it is larger, and is situated beside the high road to Tullamore. The core of the esker consists of a bedded gravel with an arched dip. The ridge is not symmetrical in section. The beds dipping south, on the southern side of the esker, are much thicker than those on the northern side, which dip north. The stratification on the northern side is more confused and irregular. The bedded gravels, which form the main mass of the esker, are covered by a bed crowded with boulders.

After the reunion of the two arms of the Clara Esker, it passes Durrow Abbey to

Newtonlow, where it bends sharply back to form the Long Hill, which is cut through by the Brosna River south of Ballynagore, and is continued, though with two gaps, through the eskers of Streamstown and Moyvore to the Inny Valley.

4. *The Athenry Esker.*

West of Ballinasloe the eskers are represented by irregular patches and mounds of drift.* Near Athenry the esker is again developed in continuous ridges, one of which is well seen from the railway to the north of Athenry Station. The structure of this esker is well exposed where it is cut through by the Tuam Railway, and in pits and road sections thence westward past Athenry toward Castle Lambert.

The section at the Tuam Railway shows the following section in descending order:—Bouldery drift, inclined southward, 10 feet; fine-grained, black chert gravel containing numerous boulders; this gravel occurs in three layers, two of which unite in the middle of the section; the two bouldery beds there end off against a tumbled mass of boulders, which form the core of the esker. On both sides the beds dip away from the middle line of the esker. Some of the boulders are striated.

A small road section to the west of this pit shows a well-defined repetition of the coarser bands, to which my attention was called by Mr. R. Durdle; and, as he remarked, this structure seems too indefinite to be a seasonal banding, though it is clearly due to variations in the strength of the currents which distributed the esker material.

On the southern slope of the esker, a little west of the last section, is a small pit, 10 feet deep, in a well-bedded coarse gravel interbedded with a much finer gravel. At the eastern end there are five layers of coarse gravel with cobbles; at the western end these have united to form only three layers. Some of the boulders in this pit are as distinctly glaciated as those in boulder clay.

At a pit further west, on the eastern side of the Tuam road, are numerous patches of sand in boulder drift. The arrangement of the material in this pit is very irregular, and the sheets of sand were probably deposited in water between the ice front and the esker.

At the quarry a little further north on the opposite side of the Tuam road the surface of the limestone is well glaciated. Upon this rests a sandy drift containing many striated and encrusted boulders. This material is identical with that sometimes described as boulder clay, though it is so sandy that the term clay for it is a misnomer. This drift does not at the quarry give rise to esker forms, though some of the sections are indistinguishable from esker drift; thus, in one place, a bed of false-bedded sand and gravel is cut off above, and is covered by a coarse morainic bouldery drift. The largest stones in this drift are striated.

The Athenry Esker in the sections from the Tuam road eastward to the Tuam

* 'Mem. Geol. Surv., Ireland,' Explanation Sheets 96–7, 106–7, p. 28 (1867).

railway is largely composed of sand and fine gravel. The sand is often as sharp as sea sand, and it is capped and interbedded with boulder drift.

5. *The Ballyhaunis and Dunmore System.*

One of the best cases of several eskers joining like the confluent tributaries of a river is supplied by the Ballyhaunis system. Thus in the Tuam district three eskers, which trend north-eastward, unite near Ballinlough (fig. 9).

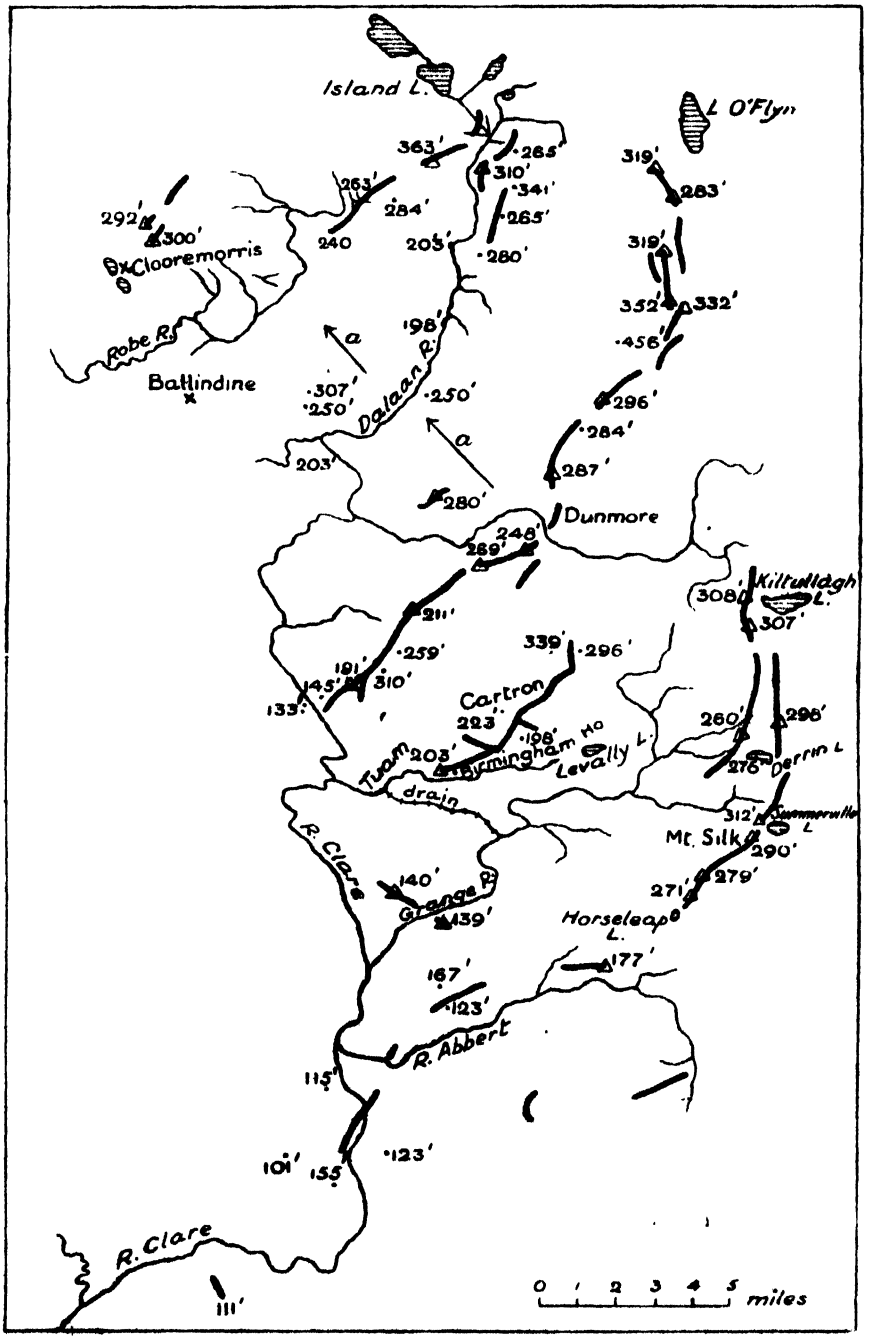


FIG. 9.—The Esker System, near Dunmore, Co. Galway. The arrows, *a*, *a*, show the course of the ice movement (after SOLLAS). Heights in feet from Ordnance and Geological Survey Maps. The eskers in this system are transverse to the course of the ice movement; they approach one another to the north, where the levels are higher than to the south. Eskers shown by thick lines. *For* Clooremorris *read* Claremorris.

Of these three eskers the southernmost follows at first the course of the Abbert River, and then crosses the divide between it and the Grange River, passing about six miles south and south-east of Tuam through Ballyglunin and Mt. Silk; it bends first northward through Raheen to the west of Kilnalag, and then north-westward to join the second member of this system.

The second esker begins a little east of Tuam, 2 miles east of which it passes Birmingham House; it is well developed 2 miles further east about Cartron; thence it trends north-eastward, and, after a long gap near Lake Makeeran, continues north through Stoneypark, and west of Cashel and Kilnalag, where it joins the two other eskers. The branching of this esker near Stoneypark is described and illustrated in the 'Mem. Geol. Surv., Ireland,' 1871, Explanation Sheets 86–88.

The third branch starts 2 miles north-west of Tuam, passes through Castletown and Dunmore, and joins the other two eskers, which continue northward towards Lough O'Flyn.

The easternmost of these three eskers lies along the watershed between the Clara and Suck Rivers. The general level of the adjacent country rises from south and south-west to north and north-east. As the ground is sloping southward if the eskers had been due to three rivers which had cut channels through a glacier down to the underlying rock, their flow should have been southward, and not in the opposite direction. On the fluviatile theory the rivers would have been divergent from the north and north-east, rather than confluent from the south-west. The three eskers, moreover, lie across the main direction of the ice flow. The distribution of the erratics, the directions of the striæ, and the trend of the drumlins, show that the main flow of the ice was from north-west to south-east.* These eskers were probably deposited along the edge of the ice sheet at stages in its retreat.

The morainic character of these eskers is shown in sections near Castletown House to the south-west of Dunmore, at Pollaphuca, at the old lime kilns beside the road junction to the south-east of Knockavenny House, and along the main road to Tuam near Birmingham House. The structure of the Dunmore Esker may be illustrated by reference to six gravel pits north-north-east of Castletown House and between it and the Dunmore road, near Grange. North of Castletown House the esker occurs in three branches, each of which has been cut through by the road. The eastern branch consists of beach material, with patches of fine gravel and scattered boulders, some of which are 2 feet long. It rests upon boulder clay, which is exposed in the low ground to the west. The westernmost division of the esker is very sandy, and is said to have yielded shells; but I did not find any.

The general character of the material in this part of the Dunmore Esker is an unstratified morainic drift. It may be described as a gravelly beach moraine. Most of the sections yielded ice-scratched stones and boulders up to 1 foot long; the

* Prof. SOLLAS recognised that the eskers near Ballyhaunis, Dunmore, and Tuam lie across the ice flow; he suggested that the position of the esker-forming rivers was determined by crevasses.

largest measured is 4 feet in diameter. The upper layer consists of a bedded sand. One of the best pits is that close by the roadside, south of Grange; in it the boulders range up to 3 feet in diameter, and are mixed with black chert, gravel, and sand; a faintly bedded character is given to the deposits by short lines of sand, but the material, as a whole, is unstratified. The irregular arrangement of the boulders and occasional bedding give this pit a morainic aspect.

The esker near Birmingham House and Pollaphuca shows more clearly the characteristics of a morainic drift, which is either unstratified or but occasionally stratified. It is therefore very different in character from ordinary fluvioglacial material. The pit in the esker just west of Birmingham House, 2 miles east of Tuam, contains boulders 2½ feet in diameter, in coarse unstratified morainic drift.

One and a quarter miles further east, a branch road to the north-east towards Cartron cuts through this esker and shows clear sections, one of which is at an old kiln just by the junction of the two roads. This pit exposes unstratified bouldery drift, cut off above by a level layer of sand resting on a line of cobbles; above this old surface, which is the only definite stratification in the pit, is a layer of boulders and cobbles. The top of this esker must have been planed down and covered by a layer of sand, upon which fresh bouldery drift was deposited.

To the north of the bog, on the road to Cartron, the esker is exposed again on the western side of the road. The most interesting feature in this pit is the presence of large encrusted boulders, some of which are standing on end. Thus, in the upper part of the pit, a boulder, which is 2 feet 8 inches high and 2 feet 4 inches wide, is standing upon its lower edge, which is only 10 inches wide. None of the boulders seen are striated.

These two members of the Ballyhaunis system are therefore not composed of fluvioglacial, but of morainic material. The Ballyhaunis Eskers were probably deposited by ice, which came from the hills of Mayo, as a series of beach moraines at successive stages in its recession to the north-west.

6. *The Ross–Gortachalla Esker.*

The Ross–Gortachalla Esker, about ten miles north-west of Galway, extends from near Ross Station eastward to Loch Corrib. It was briefly described by KINAHAN,* and SOLLAS (1896, p. 809) has suggested, as a "bare possibility," that it may belong to the Ballyhaunis system. Its course is irregular, as denudation has in places broken it into isolated mounds. A pit, south of the esker and south-east of the Roman Catholic chapel at Ross, shows that it rests on a sandy boulder clay, which contains many boulders of limestone and some of porphyritic granite. The material in the adjacent esker is a stony gravel, with the pebbles strongly faceted and not very water-worn. Sections north of the road and north of the eastern end of Ross Lake show that the esker there consists mainly of closely packed pebbles,

* 'Mem. Geol. Surv., Ireland,' Explanation Sheet 105, p. 44 (1869).

while the lowest layer exposed is of sand, with but few pebbles. The cobbles are mainly of limestone and chert, with a few of gneiss and schist, but I saw none of the granite, which is so abundant in the boulder clay. The esker here consists of the remains of two ridges, and a third is represented further to the south, on the margin of Ross Lake. A pit in the esker knoll, situated just east of the road which goes south past the eastern end of Ross Lake, exposes a typical glacieluvial gravel, which is unstratified, and has abundant pebbles of schist and gneiss, but I saw none of granite. Many of the pebbles are faceted, but are not striated.

A gravel pit near Ower contains at its northern end many limestone boulders, from 3 to 4 feet in diameter, in a faintly bedded, fine-grained sand. At the southern end of this pit the sand is coarse, sharply angular, and unsorted; it is not a river sand, but resembles a bed of sand formed as rainwash. The arrangement of the materials here suggests the former existence of a lake between the ice and the esker. South of Ower the esker is a high, narrow, irregular ridge, which is steepest to the south. Some shallow gravel pits north-west of Gortachalla Lough work a stony, unsorted glacieluvial gravel, which consists of flat-sided, subangular pebbles. They are not striated; there are no boulders, and no specimens of the coarse porphyritic granite from the hills to the south-west. There are, however, abundant pebbles of gneiss and schist derived from the area to the west and north-west. In the southern slopes of the esker, toward the eastern end of Gortachalla Lough, the stones are in places packed as closely as in a shingle beach.

The material of this Ross-Gortachalla Esker is not fluvioglacial, because it is not a river deposit; most of it is unbedded, and, though its pebbles have been so washed as to have lost any glacial striæ, they are still angular and flat-faced. Bedding and layers of sand are exceptional, though they occur where small lakes or ponds had formed between the esker and the retreating ice. The abundance of pebbles of gneiss and schist show that the material came from the west or north-west, and, if the esker had been due to a glacial river, the pebbles should become smaller to the east. This esker seems to me a ridge of typical glacieluvial materials; its composition, course, and curvature show that it was due to a glacier which flowed south-eastward from the mountains of Connemara. The esker was probably deposited along the edge of the Loch Corrib glacier during one stage of its retreat.

7. *The Greenhills Esker, near Dublin.*

The Greenhills Esker, to the south-west of Dublin,* is one of the most accessible in Ireland, and is exposed in numerous extensive sand and gravel pits. I was kindly guided to it on my first visit by Prof. Seymour. Its southern end is on the bank of the Dodder River at Balrothery. It rests in places on water-worn Carboniferous rocks, which are sometimes separated from the esker by a sheet of boulder clay.

* 'Mem. Geol. Surv., Ireland,' Explanation Sheet 112, Dublin, 1903, pp. 50–51, 97–100.

The existence of this clay has been used as an argument for the glacial-canyon theory of eskers. The esker in places shows the arched bedding, which is often claimed as characteristic of true eskers. The material is in places finer-grained on the western slope, while the bedding is steepest on the eastern front. Some of the large lenticles of gravel are coarsest to the south, but the included rocks indicate that the material came from the north or north-west—a fact previously recorded by CARVELL LEWIS (1894, p. 149). Glacial striæ are said to be not uncommon on the stones, and Prof. SEYMOUR and I noticed clear glacial grooves on the boulders in the lower part of the esker.

One of the most conspicuous features in the constitution of this esker is the abundance of laminated clay and loam, as in the deep pit at Airmont.* There the loam had been cut away in steps, and the hollows thus formed occupied by a coarse torrential gravel, which also forms the summit of the esker. The gravel often occurs in lenticular patches interstratified in the clays.

This esker is in some places a single narrow ridge, and in others a broad bank of confused interstratified sand, gravel, and clay. The material does not, as a whole, resemble the deposit in a glacial river, for the amount of clay is far greater than would be expected under such conditions.

The characteristics of this Greenhills Esker suggest that it was deposited as a wide bank of laminated sand and loam on the margin of a sheet of water; that the deposition of the finer materials was interrupted by occasional floods carrying coarse gravel; and that finally the bank was cut into by strong currents which deposited on its flanks and above it sheets of coarse torrential gravel.

The arrangement of the materials in this esker appears to indicate their deposition as a marginal bank along an ice sheet. The rise in level southward, although the material has come from the north, is incompatible with the fluviatile theory, unless the river had flowed over the glacier, and such a position appears irreconcilable with the regular bedding of the materials. If they had been first deposited on the bed of a supra-glacial river and then redeposited by the melting of the underlying ice, the bedding should have been more confused and irregular.

8. *The Eskers of Tyrone.*

A series of eskers different in several respects from those of the Central Plain occur on the moorlands of Tyrone on the divide between the Shrule, the upper part of the River Foyle, and Lough Neagh; and between the Sperrin Mountains (2,240 feet) to the north, and the hills to the north of Pomeroy. The eskers have been described in 'Mem. Geol. Surv., Ireland,' Explanation Sheets 26, 1884; 34, 1878; and 35, 1877. Some of these eskers are well exposed at Crockadoo (at the cross roads, 487 feet), at the north-western foot of Craigballyharky (771 feet), along the direct road from

* This locality is named Palatine House in the 1902 edition of Sheet 112.

Cookstown to Omagh, near Drumshambo, especially between Dunnamore Bridge (at 500 feet) and Teebane Bridge, and finally north of Creggan School (600 feet).

The eskers to the south of these hills in the valley between Pomeroy and Carrickmore appear to be much larger; they were referred to as kames by CARVILL LEWIS (1894, p. 115). He stated that the "kames" in this district, near Lough Cam, were the largest he had seen. I have chiefly examined those near the Cookstown-Omagh road, where they consist of coarse sand with many faceted but not striated pebbles. At Drumshambo they are mainly granite and greenstones, and the gravel has all the characteristics of glacieluvial deposits. The arrangement of the eskers is seen from the ridge (at 617 feet) west of Barony Bridge; and they are seen to lie along the northern foot of Evishanoran Mountain (886 feet), which is north by west of Pomeroy. The esker rises up the hill side on a sinuous course. It has obviously been formed as a marginal formation due to a glacier flowing northward and north-westward from the Evishanoran Hills. The esker near Dunnamore was probably similarly due to ice from Slievemore (842 feet). This series of eskers seems to have had a similar origin to the ridge at Polmont, in Linlithgow County, which has been described (GREGORY, 1912 (1), pp. 209–210) as a glacieluvial kame.

III. PALÆONTOLOGICAL EVIDENCE.

OLDHAM (1844, pp. 61–64, 130–132) has collected records showing that marine shells are widespread in the Irish drifts. KINAHAN found shell fragments in the esker at Maryboro*; their occurrence in the glacial drifts to the height of 1,300 feet at Three Rock Mountain near Carrickmines is well known, and the shells there were numerous and well preserved. OLDHAM records them from various localities near Dublin, including Howth, Bray, Swords (150 feet), and Finglass (200 feet), and ranging to the height of 600 feet above sea-level; in northern Ireland his records include Belfast (106 feet), Londonderry (300 feet), County Sligo (100 to 200 feet); in western Ireland, Tarmon Hill, County Mayo (250 feet); at Naas (380 feet); in an esker near Roscrea, County Tipperary, at 400 feet, "in the very centre of the island" (p. 66); also (*ibid*, p. 131) a *Buccinum undatum* in the gravels at Moate, a locality near Clara traversed by the eastern continuation of the Athlone esker.

According to OLDHAM and KINAHAN'S records marine shells have been found at three significant localities in the chief esker district of Central Ireland.

It has been suggested in conversation that these records are mostly based on shells which were carried inland for food and were dropped in the pits by man. The evidence cannot be so summarily dismissed. KINAHAN and MALLET (in OLDHAM, 1844, p. 131) were not likely to have mistaken modern shells for fossils; and as the shells at 1,300 feet above sea-level on Three Rock Mountain are undoubtedly fossil, there is no reason why marine shells should not occur at lower levels inland.

* 'Mem. Geol. Surv., Ireland,' Sheet 128, p. 30 (1859).

The shells in the esker drifts are, however, rare and fragmentary, as is only natural, since the drifts were no doubt deposited in conditions unfavourable either to the existence or preservation of mollusca. Shell fragments are friable and easily dissolved, and there would be little chance of finding them except in newly exposed material. They would probably occur only in pockets, found occasionally when the gravel pits were being worked more extensively than they are at present. The sections at Maryboro where KINAHAN found his shells are now overgrown, and the newer pits to the north are in material very unfavourable to the preservation of shells.

Although it seems necessary to accept the records it would be unwise to lay much stress on them since the shells may have been *remanié* from the underlying boulder clay. The "glacialoid boulder clay" or "semi-morainic drift" of KINAHAN is doubtless non-marine; but the lower compact boulder clay in which foraminifera are widespread, may be due, like the boulder clays in the Spitsbergen fiords, to glacier ice melting in the sea.

Looking for fossils in the eskers my attention was attracted by some calcareous encrustations in boulders near Clara and Ballinasloe, which were composed of confluent discs with lobed margins like Melobesia. Calcareous crusts are common in the eskers, and are no doubt usually tufaceous and inorganic. But some of them so strikingly resembled the nullipores which encrust the boulders on the Spitsbergen coast, that I had sections cut, and the first showed structures like those of calcareous algæ. I therefore submitted specimens to Prof. F. O. BOWER, who reported them to be "either Melobesia or some Melobesia-like organism." To obtain a more precise identification I submitted them to Dr. A. B. RENDLE and Mr. A. GEPP, of the Natural History Museum, who recommended, in order to secure the most authoritative available opinion on their nature, that they should be sent to Madame LEMOINE, of Paris. She kindly examined the material and identified the section from Ballinasloe as unquestionably a Lithothamnium.

Most of the calcareous encrustations in the eskers are no doubt inorganic, as shown by the fact that in some layers they occur only on the under surfaces; in others they have cemented grains of grit and small stones to the boulders. But Madame LEMOINE'S identification shows that some of these encrustations are algal and marine in origin. Others may have had the same formation, but have been so altered by percolating water that any organic structure has been destroyed.* The encrustations which externally resemble calcareous algæ are restricted to special layers and to the low level eskers. I saw none, for example, in the high level eskers of Tyrone; and though their absence thence might be attributed to those eskers containing little limestone, this is not an adequate explanation, as some of the stones have

* The Melobesia-like aspect of these encrustations is soon destroyed on exposure to the weather. On returning to the pit at Ballinasloe, a few months after my first visit, to collect further specimens, I found the features which had led me to suspect their organic origin had almost entirely disappeared.

tufaceous calcareous encrustations. The algal-like encrustations are absent from the eskers to the north-west of Athlone, which seem to me due to glacial rivers; and also from layers of gravel in the main eskers which appear to have been deposited by streams of water.

The objection that if any of these encrustations were of marine origin, shell fragments would occur with them does not seem conclusive, for reasons stated on pp. 145–146.

The evidence of these encrustations is, however, open to the objection that the specimens may have been derived from the underlying boulder clay, the marine origin of which has been persistently maintained, in spite of the rejection of that view by, at one time, the great majority of British glacial geologists. The boulder clay has been shown by Mr. Joseph Wright to contain widespread indigenous Foraminifera; and it seems very difficult to reject such evidence as to the marine origin of this material. If the boulder clay beneath the eskers be marine, it is possible that boulders encrusted by calcareous algæ were dropped into the eskers while embedded in frozen mud; and when the material thawed the clay would have been washed away, leaving the nullipores uninjured on the boulders. Owing to that possibility, the occurrence of Lithothamnium on the boulders is not conclusive of the marine origin of the eskers themselves.

In addition to the laminar encrustations, there are numerous tubular structures. The best preserved are small calcareous tubular branched tufts, resembling Bryozoa, which occur in hollows between the boulders in the pit east of Clara. The adjacent stones contain many encrusting tubuli, which I suspected to be the rooting fibres of Crisidia. On one of these occurred an expansion, which I sketched in the pit as the gonœcium of a Bryozoa, but this specimen was broken in transport. I showed some of these tubes to Sir Sydney Harmer, F.R.S., but he does not accept them as Bryozoa. I also showed some of them to Prof. J. S. Gemmill, to know what else they might be. Owing to their resemblance to Bryozoa, he submitted them to Mr. Alex. Gray, the most experienced authority on the Bryozoa of the Clyde estuary; he stated that, in his opinion, they are the rooting fibres of Crisidia. In view, however, of Sir Sydney Harmer's rejection of this conclusion, the nature of these tubes must be left uncertain.

Some tubular encrustations on the esker stones have been formed by the deposition of carbonate of lime around roots. Some of those near the surface may have been due to the action of grass roots in recent time, but many of the tubes were clearly contemporary with the formation of the esker, as they are found in the lower layers, which are separated from the surface by beds without these encrustations. Vertical calcareous cylindrical concretions, like those commonly found around grass roots and stems in calcareous dunes, occur occasionally, as, for example, in the Maryboro Esker.

IV. The Origin and Classification of the Irish Eskers.

The Irish eskers appear to include four different formations: (1) fluvioglacial ridges deposited along the course of glacial rivers; (2 and 3) ridges of fluvioglacial and of glacieluvial materials deposited beside the margin of melting ice sheets; and (4) banks of fluvioglacial material left by the denudation of larger sheets. The most important of the Irish eskers are the marginal formations, while the members of the first of the above four classes are comparatively few and small.

The first test whether the eskers were due to subglacial rivers or were deposited as marginal banks, is the source of the esker materials. These should have travelled along such eskers as were made, like Swedish osar, by subglacial rivers, whereas the materials of the chief Irish eskers were introduced from the side. "Drumlins," says Maxwell Close (1876, p. 211), "always seem to have been formed by something that acted along their length, whereas esker ridges seldom suggest such an idea." The available evidence as to the source of the Irish esker material is scanty, since there are few rocks in the esker district which show the direction of the ice movement. Granites and gneisses are strewn over the Carboniferous rocks near the western margin of the Central Plain and indicate a movement from west to east. But in these cases it may be argued that the rocks have been derived from the underlying boulder clay.

1. *The Trend of the Eskers and Direction of the Ice Flow.*

Prof. Sollas' map (1896, p. 805, fig. 2) represents the ice movement in the Dunmore district as from south-east to north-west. It is clear, however, that a little further south the predominant movement of the ice, which deposited the boulder clay and probably also the eskers, was from west to east, as granite from Iar-Connaught is found in the eskers near Athenry, and as Kinahan (Kinahan, 1878, p. 249; also Kinahan and Maxwell Close, 1872, p. 9) has recorded erratics of granite from western Galway scattered over the country and sometimes upon the eskers as far east as Woodlawn and Ballinasloe, and south-eastward at Eyrecourt, Portumna, Birr, and as far as Nenagh to the south-east of Loch Derg. The highlands of eastern Mayo rise to the north-west of Dunmore; hence the last ice movement in the Dunmore district would naturally have been from north-west to south-east.

Whether the ice in that district flowed from or toward the north-west, Maxwell Close and Sollas both represent it as having been at right angles to the trend of the Ballyhaunis, Dunmore, and Tuam Eskers. These ridges of loose drift could only have accumulated across the direction of the glacial movement after the ice had become stagnant. But as Tarr has remarked regarding the stagnant ice sheets of Alaska, the rivers have a very short course upon the ice; he states (Tarr, 1908, p. 97) that "no single case was found where an esker deposit was being made *on* the ice . . . the streams are short; they keep their bottoms fairly clear of *debris*; and

in a short distance they disappear to the bottom of the glacier through moulins. Certainly, under Alaskan conditions, superglacial eskers are impossible."

Apart from the Alaskan analogy the local evidence is against the glacial-canyon theory, even if it be modified by assuming the stagnancy of the ice. For as soon as the water had worked its way to the base of the ice, it would have drained down the slope of the land; whereas the course of the confluent eskers is independent of the slope, and is often in the opposite direction. Thus the eskers between Ballyhaunis, Tuam, and Newton Bellew supply, according to Prof. SOLLAS' maps, one of the best instances of eskers joining like the tributaries of a river system. South of Ballinlough, eskers trending from the south-west at Dunmore, from the south at Curragh, and from the south and south-east past Raheen and Kilnalag, all unite into one main esker. The convergence of these eskers is, however, in the wrong direction, for as shown by the altitudes marked in fig. 9, the levels are lowest at the supposed heads of the glacial streams.

The Castletown Esker begins near the Clare River, 2½ miles north-west of Tuam at about 150 feet; it rises a mile north-eastward to 194 feet. The esker near the Grange River south of Tuam is at the level of 140 feet. The south-eastern member of this series begins north of the upper part of the Abbert River at the line of about 130 feet; the level is 271 feet at Horseleap Lough, 312 feet at Mt. Silk. The northern of these eskers near Kilnalag and Ballyhaunis occur a little above the 350 contour. Prof. SOLLAS remarked that these eskers run against the slope of the ground, and suggested that the apparent convergence to the north should perhaps be interpreted as a divergence southward, the water from the Ballyhaunis district discharging seaward through radial channels. This divergence is normal in deltas and where rivers emerge on to plains. The latter condition cannot be appealed to in this district, since the land slopes, as for example along the Dunmore Esker, about 12 feet in the mile; and the course of the eskers is independent of the relief of the land that had been established before the formation of the eskers. If the divergence be explained as a delta formation, it would imply that the eskers there were formed beside the sea or a large lake.

Hence the most striking apparent case of agreement between the plan of the eskers and that of a river system fails when examined more closely. The convergence of the three esker lines towards Ballinlough may be more probably explained as due to the ice front having retreated more slowly there than along the line from Newton Bellew to Dunmore.

2. *The Variability of the Esker Levels.*

The well known variability in the esker levels is illustrated in fig. 10 for the esker system from north of Portumna to the Clara district. The Kilcrow River, which enters the north-western corner of Loch Derg, cuts through three eskers, viz., those near Killimor, at Mootbridge, and Newbridge. These three eskers unite before reaching

the Shannon. The northern or Killimor member of this esker series begins at the height of just below 300 feet, and descends to 174 feet where it is breached by the Kilcrow River. It then rises to 222 feet and 235 feet, and descends to 167 feet (with its base at about 150 feet) to the west of the Shannon. The Mootbridge Esker begins at 200 feet; it falls to 136 feet at Mootbridge, and rises to 198 feet at its junction with the third member of the series.

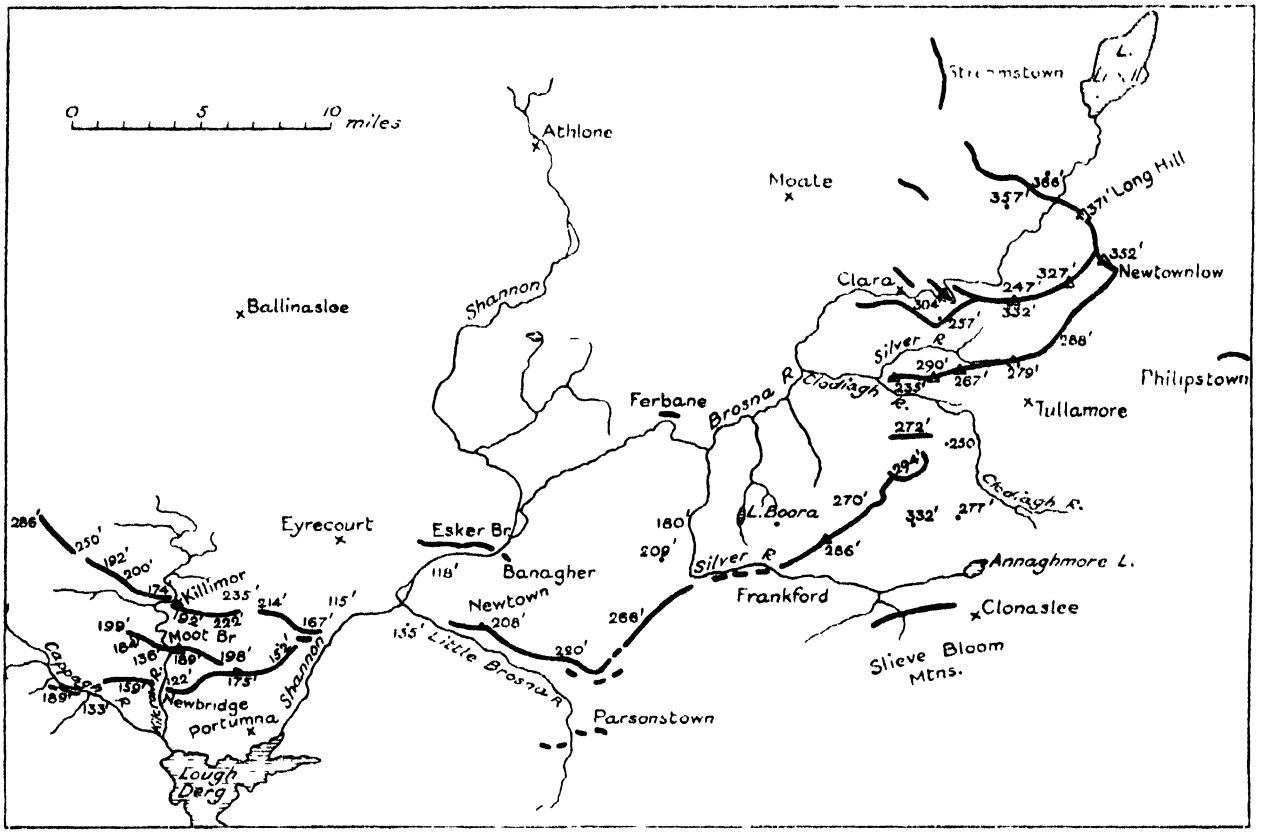


FIG. 10.—The Eskers from Portumna to Newtownlow, showing the rise and fall along their course. Heights in feet.

The Newbridge Esker also begins at about 200 feet, is at 159 feet just west of Newbridge, rises to 175 feet at its junction with the Mootbridge Esker, falls to 152 feet, and rises to 167 feet at its confluence with the Killimor Esker.

On the river theory these three eskers would have been formed by the head streams of the river which deposited the line of eskers from Parsonstown to Tullamore. That esker begins east of the Shannon, at Newtown, north of the Little Brosna River, at about 200 feet; it rises gradually eastward to 220 feet north of Parsonstown, to 266 feet at Fivealley; it is a little lower north of Frankford, and rises again at 2½ miles to the north-east, to 286 feet; 4 miles further north-east it attains the level of 294 feet, and then descends the left bank of the Clodiagh River. It resumes its course at 235 feet in the angle between the Clodiagh and Silver rivers, rises eastward to 290 feet, varies from that level to 260 feet north of

Tullamore, and rises to 352 feet at its eastern bend near Newtownlow as it approaches the eskers from Clara and Streamstown.

Of the two branches of the Clara Esker one begins at 284 feet and the other at 259 feet; the esker rises eastward to 302 feet and 327 feet. The Streamstown Esker occurs at about 350 feet; its continuation south-eastward falls to 326 feet near the Brosna, after crossing which it rises to 371 feet and 368 feet as it approaches the eskers from Parsonstown and Tullamore. The supposed outlet of the esker river from the Portumna district is, therefore, about 200 feet above the level of its head streams. This argument would not be fatal to the theory of the eskers having been formed by superglacial rivers, but that view appears to have no supporters for Irish eskers. As Prof. Sollas (1896, p. 819) observed, the structure of the Irish eskers shows that they were deposited where they are now found, and that they were not precipitated in mass by the sinking of the floor of a glacial canyon. Mr. W. B. Wright (1914, p. 39) remarks that the bedding is "frequently quite undisturbed." The levels are inconsistent with the river theory as advocated for Ireland, for if the canyons had been cut through the ice to the ground, the rivers could not have flowed in an opposite direction to the slope of the land.

It has been pointed out, as by Newberry (1878, p. 41), that some of the American eskers occupy "a topographical position, which makes it impossible that they should ever have been the beds of rivers," as they cross or even lie along the divides. The fact that the Irish eskers are independent of the slope of the country is fatal to the view that they were deposited in rivers flowing through open glacial canyons.

Observed variations in level would, on the other hand, naturally occur in marginal deposits along the edge of an ice sheet resting on an undulating surface.

3. *Esker Structures not Fluviatile.*

That the river drift theory does not explain all, or even most, of the Irish eskers is shown by their structures. Glacial rivers, such as those assumed for the formation of eskers, are naturally shallow and rapid; and in them the coarsest material should collect in the centre of the channel, while fine silts would be deposited only in the quieter water beside the banks; but in many Irish eskers the reverse is the case. Thus the core of the Ballyduff Esker consists of fine silt and clay, which is flanked and capped by coarse gravel.

Hershey (1897, p. 242) remarked that in the Illinois eskers the "beds of coarse gravel, cobbles, and small boulders are almost invariably at the top of the deposit, contrary to what we usually find in the product of ordinary stream action"; and the same objection to the fluviatile theory is applicable to many parts of the Irish eskers.

4. *The Ridge Form and the Esker Formation in Water.*

The glacial-canyon theory has the recommendation that it offers an explanation of the deposition of the esker material in long, narrow, sinuous ridges. It is true that

these ridges were once wider, for where the grain of the esker is cut off abruptly by the slope (as in figs. 3 and 4), the esker has been reduced in width since its formation. Nevertheless the ridge form was clearly one of the original characteristics of the typical esker. Their composition and structure both show that they are not ordinary moraines. They have been described as "gravelly moraines" (*e.g.*, JAS. GEIKIE, 1905, p. 312); but it seems advisable to restrict the term moraine to direct deposits from ice, and to exclude those in which material derived from ice has been carried and deposited by water. Eskers consist sometimes of fluvioglacial deposits, due to the action of glacial rivers, and of glacieluvial deposits (GREGORY, 1912, p. 210), which are due to the irregular wash of water along the edge of a melting ice sheet. The eskers, according to this nomenclature, are mainly fluvioglacial; and they are usually laid down as long banks of water-worn gravel and sand. Eskers are unknown around Alpine glaciers, and the most similar structures around Arctic glaciers occur where the ice is melting in water. The late Prof. TARR (1908, p. 97), in his account of the deposits formed on the stagnant waning ice sheets of Alaska, states that eskers are commonly found where the ice ended in bodies of standing water. He adopted the term "kames" (*ibid.*, p. 98) as distinct from eskers, and described kames as forming along the margins of the glaciers, and as attaining their best development where the gravel is deposited in lakes or on sheets of buried ice, which melts unaccompanied by deposition.

The marginal Irish eskers, according to this interpretation, agree with the great marginal bank of drift in Finland, which marks one stage in the retreat of the Finnish ice sheet. This bank, known as the "Salpausselka" (see, *e.g.*, SEDERHOLM, 1899, p. 15), it is worthy of remark, was built up where the ice sheet ended on the margin of the Yoldia Sea. Mr. W. B. WRIGHT (1914, pp. 39, 41) has already adopted the view that the Irish eskers "are more or less marginal deposits, perhaps entirely marginal," and that "the essential condition for the formation of eskers is the presence of stagnant water in front of the ice sheet during its retreat." Mr. WRIGHT regards this water as a series of local sheets of "ponded water"; he was not disposed to regard it as the sea.

5. *The Eskers a Littoral Formation.*

The members of the Irish Geological Survey who originally surveyed the eskers, especially KINAHAN, adopted the view that they were marine; and some were called "shoal eskers," on the grounds that they were formed as shoals by strong currents in a shallow glacial sea. The marine formation of the esker has been generally dismissed, but it seems nearer the truth than the later theories, which ascribed them to glacial rivers. The marine theory agrees best with their distribution. If the eskers had been formed by rivers in glacial canyons or in local bodies of "ponded water," there seems no reason for their practical restriction to the zone between the contours of 150 feet and 350 feet, and why they should not, as in

Sweden, continue to the highlands (*e.g.*, GEIKIE, 1894, p. 482).* Patches of esker-like drift occur at higher levels; they have been recorded at 464 feet in Iar-Connaught, and up to 542 feet about eight miles south-west of Roscommon; but the eskers of the typical esker district are all found below the level of 400 feet and mostly below the level of 350 feet (Map, fig. 11). They are, says HULL (1891, p. 127), "confined to the plains." KINAHAN placed the submergence beneath the "Esker Sea" as reaching the 300-foot contour (1878, pp. 225, 259, etc.) and SOLLAS (1896, p. 818) says the ground on which the eskers stand seldom rises more than 350 feet above sea-level.

The eskers range from Dublin to Galway and from Ballyhaunis as far south as Portumna, but in that wide area they do not occur above the level of 400 feet. In Tyrone, on the other hand, the eskers between Cookstown and Omagh are at the levels of from a little under 500 feet to over 700 feet (see *ante*, pp. 133–134). But the Tyrone eskers are different in character from those of the central plain; they are very irregular in level; for example, the esker which crosses the Cookstown–Omagh road, near Barony Bridge, rises rapidly up the hill side and has the aspect of a terminal moraine, formed by a glacier that came down from the Pomeroy Hills. Its material, however, is sandy and its included stones are all washed and water worn; it is a glacieluvial marginal deposit.

In the typical esker district, on the other hand, the eskers disappear or occur as irregular mounds as they approach the contour of 350 feet. Thus the eskers of the Clara and Tullamore district are separated by a gap seven miles wide from their supposed continuation in the Philipstown–Edenderry Eskers. Their absence from the high ground west of Philipstown was probably due to non-deposition, as the land is above the esker level.

The Streamstown Esker is also significant as to the restriction of eskers by the 350-foot contour. From the heights marked on the maps and a view of the country from the railway I had regarded it as an exception to the rule; but a visit showed that it lies on the floor of a valley and that the higher ground on either side is free from eskers and esker-drift. This esker may have been formed of drifts deposited in a narrow gulf or in a strait connecting the arm of the sea that extended up the Inny Valley with that from Moate to Lough Ennell.

The relation of the eskers to the 300-foot contour is illustrated in fig. 11, which shows that across the middle zone of Ireland the eskers mostly occur below the 300-foot level, though they rise somewhat above it near Ballyhaunis. The main features in the distribution of the eskers in the area illustrated by that figure are:—

(1) Their restriction, with a few exceptions, to below the level of 300 feet.

(2) That when "esker drift" occurs above that level it is in scattered mounds, the "shoal-eskers" of Kinahan; as they are not ridges they are not eskers in the restricted sense of the term.

* The restriction also of the small river-formed eskers, as at Kilbeggan, to the low ground, is due to their formation where the river mouth emerged from the ice sheet to the plain or the sea.

(3) That the eskers have a more restricted distribution than the boulder clay, which is widespread and occurs at higher levels.

(4) That the eskers belong to four main groups :

(*a*) Those of the basin of the Clare River, east of Loughs Mask and Corrib, with occasional small eskers on the western edge of that basin ; its chief members are those of the Ballyhaunis–Dunmore district.

(*b*) The eskers of the Athenry district and of the streams discharging to the eastern end of Galway Bay.

(*c*) The eskers of the Shannon basin and its tributaries, the Inny, Brosna, Suck, and Kilcrow. This group includes the eskers of Ballinasloe, Athlone, Clonmachnois, Clara, Tullamore, Streamstown, Parsonstown, etc.

(*d*) The Roscrea, Clonaslee, Mountmellick and Maryboro Eskers, which lie around the northern foot of the Slieve Bloom Mountains and of other adjacent high ground.

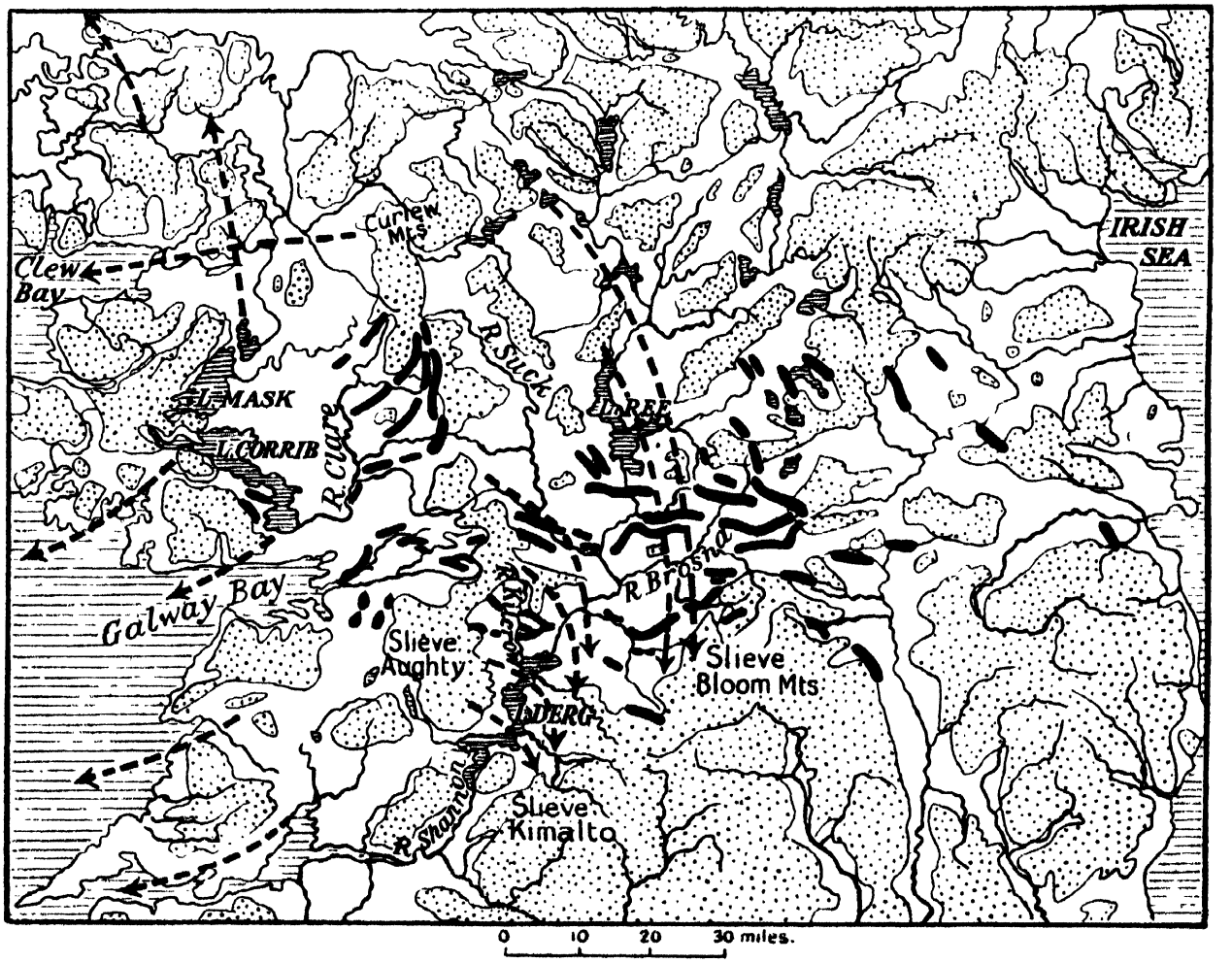


FIG. 11.—Sketch Map of the Irish Eskers in relation to the 300-foot contour; the areas above that contour are dotted ; the eskers generally end at that level. The chief exception is the north western group, which rise above 300 feet between the Clare and Suck Rivers. The broken arrows represent the course of the ice movement, after W. B. Wright ; they show that the chief eskers lie transverse to the ice movement.

Arrows indicating the ice flow are added from the map by Mr. WRIGHT (1914, p. 49), and they show that the chief eskers lie transversely to the movement of the ice. His map gives no definite directions for the Ballyhaunis district; but from the centre of ice accumulation to the east of Clew Bay and to the north-east of Lough Mask the ice flowed northward to the northern coast of Connaught, westward to Clew Bay, south-westward over Lough Corrib and western Galway. The position of the Ballyhaunis–Dunmore Eskers indicates their formation on the receding south-eastern edge of the Mayo ice sheet. Its recession left the Lough Corrib basin clear, and ice from southern Connemara was able to flow eastward past Galway to Athenry and Ballinasloe, carrying with it the boulders of granite from Iar-Connaught, which afford clear evidence of this movement. Some of this ice probably crossed the divide between Galway Bay and the Shannon, and reached Lough Derg through the valley of the Kilcrow River. This ice lobe then formed the eskers of the Killimor district, and later during its retreat those of the Athenry district.

Ice from the Curlew Mountains and other highlands of southern Sligo and Roscommon flowed south into the valleys of Suck and the Shannon, and reached the lowlands bounded to the south by Slieve Aughty, Slieve Kimalta and Slieve Bloom Mountains. Mr. WRIGHT'S arrows show that this Shannon glacier flowed transversely to the direction of the main eskers of Central Ireland; those of the eastern members of this series, such as the Long Hill near Ballygore, the Streamstown Esker, and those of the Inny were parallel to the main direction of this glacier, but they doubtless were the marginal formations along its eastern edge.

One remarkable contrast between the eskers of the Clare River and of the Shannon is that those of the Shannon basin are further to the south, which suggests that the Shannon glacier lasted longer than that which flowed into the Clare basin from Mayo, so that the emergence of the country prevented the formation of eskers during the recession of the ice from Lough Ree northwards. The dilapidated fragmentary condition of the western ends of the Ballyhaunis and Dunmore Eskers is consistent with their formation somewhat earlier than those in the Shannon basin.

The Roscrea, Clonaslee,* Mountmellick, and Maryboro Eskers were probably formed by ice which flowed down the northern slopes of the Slieve Bloom Mountains.

This sharp vertical limitation of the eskers is in favour of their formation having been controlled by a sheet of water, and this must have been either the sea or a glacial lake.

The difficulty in the assumption of a glacial lake is to account for the containing barriers. There is no difficulty regarding its northern and north-western margin, for that would be formed by the ice sheet itself. Partial boundaries would have been provided to the south-west by the Slieve Aughty Mountains, to the south by the Silvermines Mountains, and to the south-east by the Slieve Bloom Mountains; but these hills would leave wide gaps below the level of 350 feet, and even below 250 feet

* The Clonaslee Esker ranges up to 390 feet.

at Loughrea, Scarriff (140 feet), and the Nenagh–Curraheen gap (247 feet), while to the east of Tullamore there were wide tracts below the level of 350 feet. Unless all these outlets had been blocked by ice, there could have been no glacial lakes adequate for the formation of the eskers.

The extension of the sea across the esker plain presents no such difficulties. Some submergence of the British area during glacial times is now generally accepted; and even if this submergence were inadequate for the deposition *in situ* of the shells at 1300 feet on Three Rock Mountains south of Dublin, it was sufficient for the sea to have covered the Irish plain. Even the late CARVELL LEWIS, a most resolute opponent of the view that the British Isles were submerged in glacial times to the contour of about 1300 feet, accepted a submergence of 400 feet to the west of Dublin; and that amount would give the sea the range in Ireland required to explain the deposition of the eskers in a glacial sea.

The Irish eskers do not appear to be ordinary beach deposits, though KINAHAN at one time ('Mem. Geol. Surv., Ireland,' Explanation Sheet 128, 1859, pp. 29–30) compared them to raised storm beaches, in which the material is piled up with an arched dip down both slopes. The rejection of this hypothesis is not dependent on the absence or rarity of marine shells; for though OLDHAM'S paper (1844) shows that marine shell fragments are widely distributed in Ireland, they are certainly rare in the eskers. The absence of shells from these gravels does not, however, disprove their marine origin; for, as was pointed out by BROWN (1870, pp. 638, 688), many parts of the Greenland fiords, owing to the disturbed and muddy condition of the water, are "bare of marine life" and "unfavourable for sea-animals"; hence the deposits in them would be unfossiliferous. From the character of the esker materials any shells that might have been present would have been destroyed during its formation or dissolved subsequently.

6. *The Rarity of Fossils.*

The poverty of the marine fauna in both the Boulder Clay and the eskers is not surprising on the assumption that they were deposited in an ice-covered sea. NANSEN (1902, pp. 422–3) has pointed out that where the Arctic Sea is ice covered, owing to the absorption of light very few plants live in it. "We found," he says, "extremely little plant-life in the interior of the North Polar Sea, and it is as a rule in vain that Mr. H. H. GRAN has searched our plankton samples for diatoms." The poverty in plant-life involves the rarity of animal life. NANSEN refers to the extreme poverty of the plankton life in the Arctic Ocean (*ibid.*, p. 423; also vol. 4, 1904, p. 221), and to the unusually small amount of matter originating from organisms in the oceanic deposits. He says that it was "in most cases extremely difficult to find traces of shells of Foraminifera or other organisms," and that "I hardly found any Foraminifera in the Plankton samples taken in the North Polar Basin." He therefore attributes the few Foraminifera that were found to deposition when "the biological conditions

of the North Polar Basin were more favourable than they are now" (*ibid.*, vol. 4, p. 221).

As remarked by SCOULER (quoted OLDHAM, 1844, p. 64), the eskers were deposited "in shallow but turbulent waters, little favourable for the abode of molluscous animals." Even on open beaches in the Antarctic shells appear to be exceptional. According to Mr. JAMES MURRAY, the biologist with Sir ERNEST SHACKLETON'S first expedition, the action of the shore ice prevents the accumulation of shell beaches in the Antarctic. He states (1910, p. 2) that "on the shore there is no vestige of marine life, animal or vegetable, such as is found in the littoral zone of other coasts." "The presence of an ice-foot throughout the greater part of the year, and the grinding of ice along the coast where there is open sea, must destroy any living things which attempt to establish themselves. The zone thus kept devoid of life is of no great depth." HEDLEY (1916, p. 85) has remarked that the shell-bearing "raised beaches" of Macmurdo Sound are not shore beaches, but are probably upheaved from a deeper horizon.

If the eskers were deposited when the sea extended over Central Ireland to the contour of between 300 and 350 feet, the esker area would have been covered by a shallow bay, connected to the outer sea by narrow straits to the south-west and south, and with broader but still shallow channels to the east and south-east. This bay would have been occupied by ice-cold muddy water, swept by strong tides, and constantly churned up by waves from the calving ice front. The conditions would have been most unfavourable to animal life. Though the occurrence of shells would therefore be exceptional, they have been recorded in the eskers of Naas (380 feet) to the east, at Maryboro to the south-east, at Roscrea (400 feet) to the south, and at Moate (approximately 250 feet) in the centre of the esker area.

Madame LEMOINE'S determination of the nullipore from Ballinasloe is therefore consistent with the records of marine shells in the eskers of that part of Ireland.

7. *Definition of the Term Esker in Glacial Geology.*

When MAXWELL CLOSE (1867, pp. 211–212; see also KINAHAN and CLOSE, 1872, p. 6) separated drumlins from eskers, he accepted the latter term for fluvio-glacial ridges and mounds. In 1912 (No. 2), I summarised the history of the term kame and esker, quoted a series of authorities as to their usage, and followed Prof. T. C. CHAMBERLIN (1883, p. 300; renewed in 1899) in adopting the term kame for marginal fluvioglacial formations and osar or eskers for fluvioglacial ridges which are due to glacial streams, and occur on lines parallel to the flow of the ice. That definition, which has been often adopted in America, and Prof. SOLLAS'S interpretation of the Irish eskers, would restrict the term esker to fluvioglacial ridges deposited along glacial rivers. It would be synonymous with osar. The Irish eskers, however, include several varieties of fluvioglacial and glacieluvial ridges, and also mounds, and, if restricted to osar, the term would be limited to the least

extensive and important variety of eskers. G. H. STONE has proposed (1899, pp. 359–360) to "employ the term esker as a general term applicable to any mass or ridge of gravel irrespective of genetic classification," and that proposal is consistent with the Irish use of the term. The term osar was adopted by JAS. GEIKIE (1894, p. 169 ; spelt åsar) in preference to esker, and STONE accepted it in the same restricted sense. It seems best to follow their example and use the term esker in its original meaning for Irish ridges and mounds composed of glacial sand and gravel, and adopt osar, as the approximately phonetic rendering of åsar, for fluvioglacial ridges formed along glacial rivers. The term kame is then applicable, in accordance with general usage, for the marginal formations. According to this terminology, however, the major Irish eskers are kames.

The following classification summarises the structure of these drift formations as thus defined :—

(I) Drumlins—mounds composed of boulder clay.

(II) Osar—fluvioglacial ridges formed of sand and gravel deposited along the course of a glacial river and typically showing a transverse seasonal banding. Irish examples are those at Kilbeggan, near Clara, and the north-west of Athlone.

(III) Kames—ridges or mounds of sand or gravel deposited by water on the margin of a melting ice sheet. They consist either of fluvioglacial material where deposited by well defined streams and rivers ; or of glacieluvial material where deposited by the wash of water down the margin of an ice sheet or by water welling up along the margin of an ice sheet.

V. SUMMARY OF CONCLUSIONS.

The Irish eskers belong to four groups.

I. The typical eskers on the Central Plain are kames, or marginal formations, deposited on the receding edge of an ice sheet. They are not moraines, as their materials were laid down by water and not dumped directly from melting ice. They were not formed along river beds, as adjacent segments vary greatly and irregularly in composition, patches of false-bedded sands and gravels alternating with coarse washed morainic drift ; the false-bedding and the variations in the composition of the eskers indicate that the material came from the side of the esker and was not carried along it. The predominant material in some eskers is a bouldery drift, which has been so washed that the ice-scratches have been destroyed ; in such places the material is glacieluvial.

That the main eskers were marginal is also indicated by their relation to the drumlins, of which the longer axis is regarded as lying in the direction of the ice flow. The main eskers are transverse to the drumlins.

The main eskers are also transverse to the ice movement as indicated by the striated rock surfaces and the distribution of the erratics where the rocks give evidence as to the direction of the ice movement. The main eskers lie between the levels of 100 feet

and 350 feet; they occasionally reach 400 feet. Their abrupt upward limit indicates that their range was controlled by some agent which was widespread over the plain of west central Ireland, but had a limited vertical range. These conditions are best provided by a sheet of water; and the melting of the ice as it entered a wide sheet of water best explains the heaping up of its material into a ridge instead of its deposition as a widespread sheet.

That this sheet of water was the sea is most probable for the following reasons:—

1. The underlying boulder clay and associated drifts contain foraminifera and marine shell fragments; these range up to 1,300 feet on Three Rock Mountain and appear to be widespread at lower levels.

2. Encrustations formed on boulders at Ballinasloe are identified by Madame LEMOINE as Lithothamnium.

3. The poverty of organisms is explicable by the present distribution of life under analogous conditions in the Arctic and Antarctic seas.

4. It is now generally agreed that large parts of the British area were submerged in glacial times to a sufficient height to have flooded the typical esker district of Central Ireland.

5. The alternative that the water was a glacial lake is improbable, as the esker sea was probably in existence after the ice had receded from the southern part of the central plain; and there would be no available southern margin for the lake.

II. A second type of esker consists of banded eskers lying within and transverse to the main marginal eskers, parallel to the direction of movement of the ice. They were probably deposited as delta deposits at the mouths of glacial rivers, and are thus equivalent to the Swedish osar.

III. In Tyrone, on the hills to the north and north-west of Pomeroy, is a third group of eskers, formed as glacieluvial marginal deposits around glaciers flowing from the hills.

IV. A fourth type consists of irregularly distributed mounds of fluvioglacial drift, as west of Athlone, which are probably due to the denudation of sheets of glacial sand and gravel.

The restriction of the term esker to any one of these four groups would be inconsistent with its original meaning. The term has been often defined so as to include only eskers of the second group, which in Ireland are relatively unimportant. The most typical eskers are those of the first group; they are kames, according to the widely adopted definition which uses the term kame for marginal formations. To avoid the use of the term esker with different meanings in geology and geography, its use is recommended as a general term for Irish ridges and mounds of glacial gravels and sands, and that of osar for fluvioglacial ridges which have been formed along the courses of glacial rivers.

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V.—*The Thermo-elastic Properties of Muscle.*

By A. V. HILL, *F.R.S.*, and W. HARTREE.

(From the Physiological Laboratory, Cambridge.)

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(1) INTRODUCTION.

The thermal effects of applying a stress to a body were first studied by WEBER (1), who found that when an iron wire was stretched a thermal effect was produced, and that the thermal change was proportional to the stress. Lord KELVIN (2) deduced the general equations of thermo-elasticity from the laws of thermodynamics, and proved that with stresses of the most general type the thermal effect is proportional to the applied stress, provided the material remains perfectly elastic. In a body which expands on being warmed, the effect of an extension is a *fall* of temperature, while a body which shortens on being warmed will show a *rise* of temperature on extension. It will be remembered that ENGELMANN found that some substances (*e.g.*, catgut) containing doubly refractive particles contract on heating; such bodies, therefore, would be expected to show a rise of temperature on being loaded, and the experiments given below prove that muscles and rubber belong to this class.

The phenomena of thermo-elasticity have been employed to demonstrate the stresses set up in structures on loading them; all that is necessary is to place the "warm" junctions of a suitable thermopile against the part it is desired to investigate, and then, on loading the structure in any desired manner, the magnitude and sign of the stress in that part can be determined at once from the deflection of a galvanometer. This method, which is quite reasonably simple to employ, appears to deserve a wider application than it has received. It could be applied to a variety of substances under a variety of conditions, *e.g.*, to the materials employed in the construction of aircraft, ships, guns, etc., in which considerable stresses have to be borne by structures which have to be kept as light as possible. It might even be employed in physiology to determine the distribution of stresses in the skeleton, and

it would certainly be of interest to make an extensive investigation of the thermo-elastic properties of elastic colloidal materials.

A further application is that of determining the elastic limit of a material. When an ordinary steel wire is stretched there is a fall of temperature; as the load is increased the fall of temperature increases at a proportional rate; at the elastic limit, however, the cooling is replaced by a warming due to the work done by the load being transformed irreversibly into heat instead of into elastic potential energy. The load at which this change occurs corresponds to the elastic limit of the wire.

Various applications of the method to engineering problems have been described by COKER (5 and 6).

It would seem that the employment of the light and sensitive thermopiles now obtainable, together with photographic recording as used in our experiments, might considerably increase the variety and importance of such applications. It should be remembered that metals and many other materials can bear enormously greater stresses, and so give greater thermal effects than can muscles or rubber, so that the high sensitivity of the galvanometer required in the experiments described here could be exchanged for a more rapid response, enabling a very accurate time-record of the thermal effects to be made. A more rapid response also could be secured by employing thinner insulation on the thermopile.

Our attention was first called to the subject by Mr. C. C. MASON, of the Cambridge and Paul Instrument Company, and it seemed desirable to investigate the phenomena of thermo-elasticity in living tissues. Assuming that (like catgut) a muscle shortens on warming, one would expect that on loading it there would be an evolution and on unloading an absorption of heat. These phenomena we have observed, but the results of our experiments are much more complex than those found in the case of an ordinary elastic body such as a steel wire, owing to the peculiar elastic properties of the muscle. The experiments throw considerable light on these elastic properties—which belong not only to muscles, but to many kinds of material—and, moreover, the purely physical thermal phenomena described here, if unheeded, may considerably interfere with or distort the physiological thermal changes associated with muscular activity.

Exactly similar results have been obtained in live and in dead muscles, and analogous results in the case of a rubber band, and it appears certain that the phenomena described are purely a consequence of the elastic properties of the material, and in no way dependent on the life or structure of the muscle.

The elastic property of a muscle which differentiates it from a steel wire is that, for any load, although well within the elastic limit, the muscle does not immediately attain its new length when the load upon it is changed. For example, when a weight is hung on a muscle, the muscle continues stretching for some time; when the load is removed, it returns only slowly to its original length. The same property is true of a jelly, or of a piece of rubber, and, of course, is well known to all who

have studied the properties of materials. The implications of it, however, are peculiar, and lead to complex and interesting thermal effects of applying a stress to the body concerned. The elastic property described is presumably due, in the case of a muscle, to the existence of an elastic network, colloidal or otherwise, containing a viscous fluid in its interstices. The final equilibrium value of the extension caused by a given load is presumably a characteristic of the network. When, however, the load is suddenly altered, the viscous fluid has to change its position within the network. and, if the alteration of load is rapid, the new equilibrium length is not attained until the viscous fluid has had time to reach its new position. Consequently, if the material be unloaded and allowed to shorten rapidly, doing work, the work done will be less than the work put into it in stretching it, and the difference between the two will cause an irreversible production of heat, complicating the reversible changes predicted by the thermodynamical reasoning.

The thermal effects of passive lengthening or shortening are by no means small. They are not as large as those of stimulating a live muscle, but they are large enough to afford a notable complication in the case of any contraction in which the muscle is allowed to shorten. A discussion of these complications is given below: one obvious means of avoiding them, and the one which—fortunately, though for other reasons—has been adopted by us in most previous work, is that of making the contraction isometric. In any experiments, however, in which a muscle is initially stretched, and allowed to shorten on excitation, the purely *physical* thermal effects consequent on the shortening and the subsequent extension during relaxation, must necessarily be superimposed upon the *physiological* thermal effects attending the chemical reactions set up by the stimulus.

(2) Methods and Results of Thermal Experiments.

The instruments employed have been those described elsewhere (3). The use of the combined muscle-chamber and thermopile, immersed in well stirred water in a large double-walled vacuum flask, has ensured an absence of differences of temperature at different points on the muscle which were the stumbling block in all previous experiments in which the muscles were allowed to move over the junctions (4). The uniform temperature of the whole thermopile and muscle eliminated the possibility of a movement bringing cooler or warmer parts of the muscle on to the junctions. The muscles (a pair of sartorii from *Rana temporaria*) were fitted on the thermopile in the chamber, and a long thread taken from their upper ends, through the tube holding the chamber, up to one arm of a pivoted lever; to the other arm of the lever was tied a thread carrying a pan, in which were placed the weights required to load the muscle. The lever itself had a small weight, usually of the order of 5 grm., fixed directly to it, to provide a small constant tension on the muscle. The loading or unloading of the muscle was carried out by hand, by gently raising or lowering the pan in which the weights

were placed. For example, if it were desired to investigate the effects of loading the muscles with 60 grm., the weights were placed in the pan and the pan held in the hand with the thread attaching it to the lever loose; the muscle was then subject only to the load of the 5 grm. providing the small constant tension. When all was ready, and the photographic arrangements running, on a given signal, recorded on the photographic paper, the pan, with its weights, was gently lowered so as to hang on the lever, and subject the muscle to an additional tension of 60 grm. The deflection of the galvanometer was recorded on the paper and the record allowed to run as long as required, usually for about 30 seconds. To investigate the effects of *unloading*, the load was allowed to hang on the lever for some time, say 2 or 3 minutes, until the length of the muscle had become appreciably constant; on a given signal, the pan with its weights was gently lifted, and the photographic record made as before. Typical records are shown in fig. 1. It should be noted that the load was lowered *gently* on to the muscle and gently removed, particular care being taken to avoid anything in the nature of a jerk.

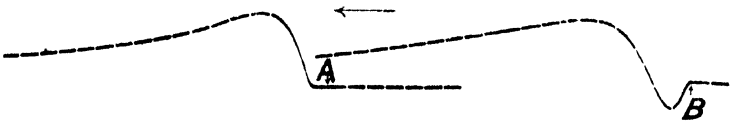


FIG. 1.—Pair of sartorius muscles from *Rana temporaria*. Permanent load 5 grm. At A on the left hand curve 155 grm. was hung gently on the muscle: the temperature rose. At B on the right hand curve, after the 155 grm. had been hanging on the muscle for some time, it was gently removed: the temperature fell rapidly (the reversible effect) and then rose (the irreversible "viscous" effect) and finally fell again (the physical loss of heat by conduction). Time in secs. shown on the curves. N.B.—These and all other records given here read from right to left.

In order to obtain satisfactory records, it was usually desirable to increase the sensitivity of the galvanometer to about $1\frac{1}{2}$ times that employed in the case of experiments on the heat-production of stimulated muscles.

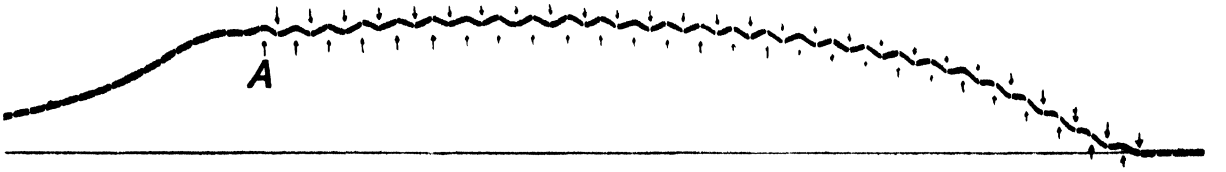


FIG. 2.—Dead muscles at 11° C. 163 grm. load "on" and "off" at intervals of 1 sec. (*i.e.*, load on—1 sec. interval—load off—1 sec. interval—load on—etc.). 50 ohms in galvanometer-thermopile circuit to reduce sensitivity, which in this experiment was about 0·26 of that in the experiment shown in fig. 1. Notice (*a*) the rise of temperature on loading followed by (*b*) the fall of temperature on unloading; superimposed upon (*c*), the gradual steady rise of temperature to a maximum (determined by a balance between heat produced and heat lost). Of these (*a*) and (*b*) are the reversible thermodynamic effects; (*c*) is the irreversible effect due to the viscous flow accompanying extension and shortening. The arrows above the curve indicate the moments at which loading occurred: the arrows below the curve indicate the moments at which unloading occurred. Time marks in secs. on the curve. At A the muscle was unloaded finally, and after a short delay began to cool down by simple loss of heat.

Various other procedures were adopted to illustrate the phenomena. For example, in some experiments the pan containing the weights was raised and lowered gently at regular intervals of, say, one second. Fig. 2 is a record of the thermal effect of such a procedure. In other experiments the muscle was loaded and then unloaded after a certain definite interval, the effect of which is shown in fig. 3.

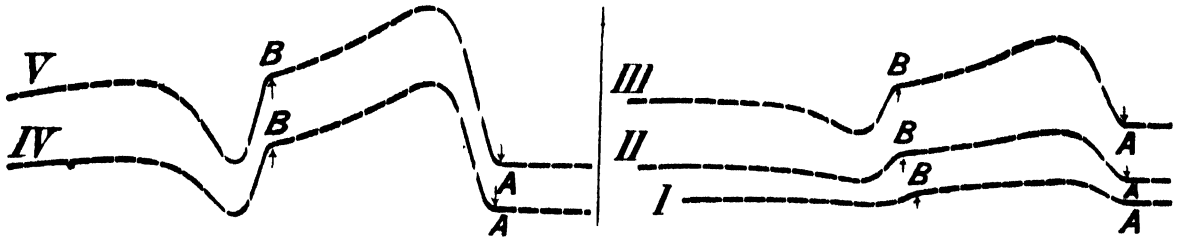


FIG. 3.—Dead muscles at −0·6° C. Various loads "on" and "off" at an interval of 10 secs. At A on each curve the load was put "on," at B it was taken "off." Curves I–V show respectively the thermal effect of loading and unloading with 20, 50, 95, 155, and 200 grm. Time marks in secs. on the curves. Notice (*a*) the sudden rise of temperature on loading, and (*b*) the sudden fall of temperature on unloading, followed by (*c*) a subsequent rise. The shape of the curves is determined largely by loss of heat through conduction. Note also that the thermal effect increases as the load is increased, but not quite proportionally.

Exactly similar results were obtained with live and with dead muscles, and there is no evidence to show that the phenomena are in any way connected with the life or mechanism of the muscle. To make the more certain of this, the observations were repeated with a narrow strip of an old Dunlop rubber tyre (inner tube), more or less of the shape and size of a pair of muscles, and lying upon the junctions of the thermopile in the same way. In order to make the motion of the rubber over the junctions more free, a drop of paraffin oil was placed upon the thermopile. The phenomena shown by the rubber were similar in type to those shown by the muscle, though quantitatively different. Fig. 4 shows the characteristic effects of loading and

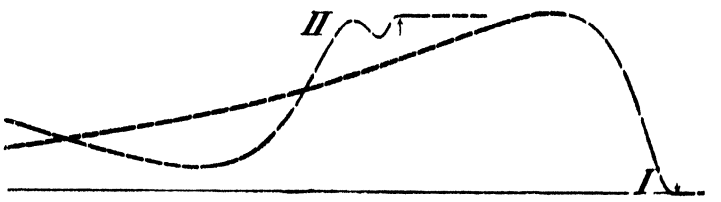


FIG. 4.—Strip of old rubber tyre, 3·5 cm. long, mass 0·59 grm., of the general shape of a pair of sartorius muscles, placed upon the thermopile in the muscle chamber at 11° C. Permanent load 150 grm. Time shown in secs. on the curves. Curve I shows the rise of temperature on loading the rubber with a further 550 grm. Curve II shows (*a*) the fall; (*b*) the rise; and (*c*) the subsequent further fall of temperature on removing the 550 grm. NOTE.—In curve I an extra 25 ohms was put in the galvanometer-thermopile circuit in order to reduce the sensitivity; consequently Curve I is on 2·4 times the scale of Curve II.

unloading the rubber. In this case there was a permanent load of 150 grm. on the rubber, and an extra load of 550 grm. was put on and taken off. Fig. 5 shows the

effects of loading followed by unloading at various intervals—1, 2, 4 secs. and "infinity."

The sensitivity, expressed in degrees Centigrade per millimetre deflection, was

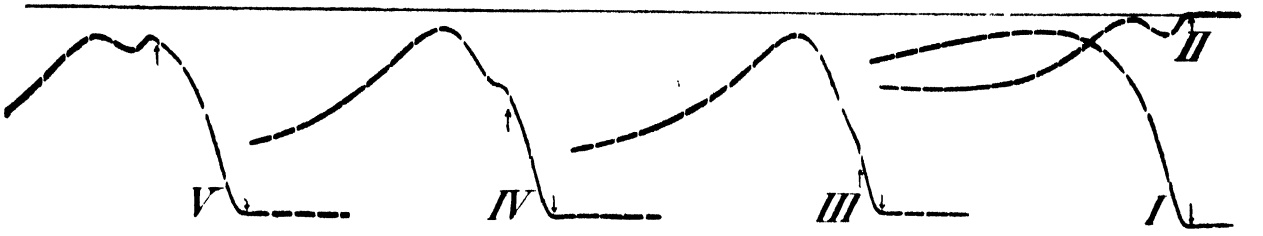


FIG. 5.—Strip of old rubber tyre, as in fig. 4. Permanent load 150 grm.

Curve I shows the rise of temperature on loading with a further 300 grm.

Curve II shows (*a*) the fall; (*b*) the rise; and (*c*) the subsequent further fall of temperature on removing the 300 grm., after it had hung on the muscle for an "infinite" time.

Curve III shows the effect of loading with 300 grm., and then unloading again after 1 sec.

Curve IV shows the effect of loading and unloading again after 2 secs.

Curve V shows the effect of loading and unloading again after 4 secs.

By an analysis of the curves of fig. 5, taking the deflections of I and II at every 1 sec. and then adding them together algebraically with an interval ("phase difference") of (*a*) 1 sec., (*b*) 2 secs., and (*c*) 4 secs., it is found that as regards the *initial* shape of the curve—*i.e.*, up to 5 or 6 secs.—there is good agreement between the results so calculated and the observed Curves III, IV, and V respectively. Afterwards, however, in all the later stages, much less heat is actually given out than would be calculated from the addition of I and II with the appropriate "phase difference": in fact—as would be expected—the irreversible processes leading to a loss of potential energy and the production of heat, are much less in extent when the load is left on for a shorter time. The same thing is shown by the fact that the total heat production in the complete cycle of loading and unloading, given very nearly by the areas of the Curves III, IV, and V, obviously increases as the interval between loading and unloading increases.

determined in the case of the experiments on muscles by means of a direct calibration, as described in another paper (3). It was about the same in the various experiments on muscles and may be taken to be approximately (in the original records)

$$1 \text{ mm.} = 6 \times 10^{-5^\circ} \text{ C. or } 0{\cdot}00006 \text{ calories per grm.}$$

One is, of course, working to a high sensitivity in these thermal measurements, as the records are reliable to about 0·1 or 0·2 mm., which means that one is reading to about 10 millionths of a degree. A less sensitive arrangement would not show to sufficient accuracy the rather small quantities involved.

[The curves as reproduced in figs. 1 to 5 have been reduced to about 40 per cent. of their original size.]

In the case of the experiments on the rubber strip a direct calibration was not possible, as the rubber is a non-conductor of electricity, and so no warming current could be applied to it. We may, however, assume for the purposes of a rough calculation of the coefficient of thermal expansion that the sensitivity in degrees Centigrade was the same as in the muscle experiments.

The sensitivity of the arrangement may be expressed also in the following form, by employing the mechanical equivalent of heat:—

1 mm. = 2·5 grm.-cm. of work per gramme of muscle.

This means that, for a pair of muscles weighing 0·16 grm., 1 mm. deflection is given when 0·4 grm.-cm. of work is absorbed by the muscles and degraded into heat. A full deflection of 6 cm. could be produced, therefore, by the absorption of mechanical work to the extent of 24 grm.-cm. This is a more expressive way of stating the sensitivity, as it is clear that 24 grm.-cm. is of the same order of size as the work done in stretching the muscle 1 cm. with a load (say) of 100 grm., whereas 0·0036 calories has at first sight no obvious relation to the same quantity.

In most of the experiments the thread connecting the muscle to the lever was free in the tube; in a few of them, however, the narrow part of the tube (about $\frac{3}{4}$ mm. diameter) was filled with vaseline and the thread moved in the vaseline. The vaseline had the effect of reducing the irreversible rise of temperature following the reversible fall of temperature caused by unloading the muscle. This fact is of interest in confirming the theory as given here, and will be discussed below.

(3) Discussion of Thermal Experiments.

Since the phenomena described are susceptible to some degree of an exact treatment by the methods of thermodynamics, it may be well to put on record here a deduction of the equations for a finite extension of an elastic string. We have thought it better to employ the conception of "free energy," rather than that of "entropy," as being more intelligible to the average reader. The principle involved is to calculate A, the free energy, *i.e.*, the maximum work which could be done by an extended string, and then, by the second law of thermodynamics, to equate dA/dT to Q/T, where T is the absolute temperature and Q is the heat absorbed in the process. If the coefficient of thermal expansion be known, dA/dT can be calculated, and therefore Q is obtained directly from the equation

$$Q = T\frac{dA}{dT}. \tag{1}$$

Let an elastic body shorten from length l_1 to length l_2, doing work, and suppose the length is l for a tension P. Then the free energy of the change is

$$A = \int_{l_2}^{l_1} P dl.$$

Differentiating with respect to T

$$\frac{dA}{dT} = \int_{l_2}^{l_1}\left(\frac{\partial P}{\partial T}\right)_l dl, \tag{2}$$

where $\left(\frac{\partial P}{\partial T}\right)_l$ is the rate of change of P with respect to T when l remains constant.

Now l is a function of two variables, and of two variables only, P and T: hence we may write, when l remains constant,

$$\delta l = 0 = \left(\frac{\partial l}{\partial P}\right)_T \delta P + \left(\frac{\partial l}{\partial T}\right)_P \delta T$$

from which

$$\left(\frac{\partial P}{\partial T}\right)_l = -\left(\frac{\partial l}{\partial T}\right)_P \Big/ \left(\frac{\partial l}{\partial P}\right)_T. \tag{3}$$

Now, if the coefficient of thermal expansion α be independent of the tension P, we have

$$\left(\frac{\partial l}{\partial T}\right)_P = \alpha l. \tag{4}$$

In this case, from (2), (3) and (4), we have

$$\frac{dA}{dT} = -\int_{l_2}^{l_1} \alpha l \left(\frac{\partial P}{\partial l}\right)_T dl.$$

Integrating by parts

$$\frac{dA}{dT} = -\alpha (P_1 l_1 - P_2 l_2) + \alpha \int_{l_2}^{l_1} P dl.$$

Hence from (1) above, the heat absorbed is given by

$$Q = \alpha T [\text{work done} - (P_1 l_1 - P_2 l_2)]. \tag{5}$$

This is the most general form of the result but, if we may assume that the body is perfectly elastic, we may write

$$\text{work done} = \tfrac{1}{2}(P_1 + P_2)(l_1 - l_2)$$

in which case

$$Q = -\tfrac{1}{2}\alpha T (P_1 - P_2)(l_1 + l_2). \tag{6}$$

In other words, when the tension is diminished heat is *evolved* equal to

$$\alpha T \,(\text{diminution of tension})\,(\text{mean length}),$$

and when the tension is increased, heat is *absorbed* equal to

$$\alpha T \,(\text{increase of tension})\,(\text{mean length}).$$

It should be noted that here we have measured everything, including the heat, in dynamical units; if the heat be measured in calories, the tensions in grams-weight and the lengths in centimetres, the heat absorbed becomes

$$\frac{\alpha T \,(\text{increase of tension})\,(\text{mean length})}{4{\cdot}24 \times 10^4} \text{ calories.}$$

If we consider the fall of temperature in an adiabatic extension instead of the heat absorbed in an isothermal one, the fall of temperature $(-\delta T)$ is given by

$$-\delta T = \frac{\alpha T \,(\text{increase of tension})\,(\text{mean length})}{(\text{mass})\,(\text{specific heat})\,4{\cdot}24 \times 10^4}. \tag{7}$$

In the case of most substances α, the coefficient of thermal expansion, is positive and of the order 10^{-5}. In the case of muscle and of rubber it appears to be negative, but of the same order of size. With these substances there is an initial rise of temperature on increasing, and an initial fall of temperature on decreasing, the tension.

We have assumed in the preceding argument that we have to deal with a perfectly elastic body undergoing a reversible change. In the case of a substance like muscle the change will be reversible only if carried out infinitely slowly, for otherwise an irreversible degradation of potential energy into heat under the action of viscous forces will occur. This matter also can be treated by means of thermodynamical reasoning. In fig. 6 are shown curves relating the extension of a muscle to its

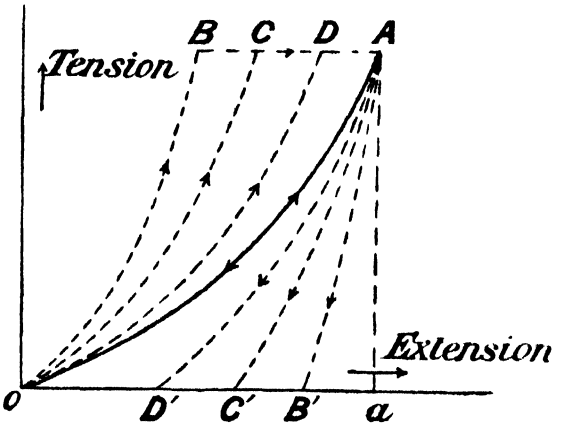


FIG. 6.—Relation between tension and extension in a muscle. The full curve OA corresponds to a very slow process of unloading or loading the muscle from or to a given tension, and represents a "reversible" process. The dotted curves represent "irreversible" processes carried out more or less rapidly. The curves OBA, OCA, and ODA correspond to *loading* carried out rapidly, the most rapid being OBA, and the least rapid ODA. The curves AB'O, AC'O, AD'O, correspond to unloading carried out rapidly, the first being the most and the last being the least rapid. The potential energy possessed by the stretched muscle corresponds to the area OAa: the work done in stretching it rapidly along (say) the curve OCA corresponds to the area OCAa: the work obtained from it on unloading it rapidly corresponds (say) to the area AC'a: the work lost and degenerated into heat irreversibly in the complete cycle corresponds therefore to the area OCAC'.

[NOTE.—The curves are illustrative only and do not represent an actual observation.]

tension. The curve OA represents the relation of extension to tension when the tension is increased exceedingly slowly from the value zero to the value Aa, and the work done in extending the muscle is represented by the area OAa. The process of passing from O to A or from A to O is strictly reversible, and no work or potential energy is degraded into heat in the process. If, however, the tension be increased more or less rapidly from zero to the value Aa, the relation between extension and tension follows one or other of the curves OBA, OCA, or ODA: OBA representing the more rapid change and ODA the less rapid. In the case of the rapid change corresponding (say) to OCA, the work done in stretching the muscle corresponds to the area OCAa, which is greater by the area OCA than the *greatest* amount of work which can be recovered on allowing the muscle to shorten again. Hence in this rapid stretching the work done re-appears in the muscle, partly as mechanical potential energy corresponding to the area OAa, and partly as heat corresponding to the area OCA. The more rapidly this stretching process is carried out the greater will be the

amount of work re-appearing irreversibly as heat in the muscle. The irreversible transformation of work into heat is presumably caused by the rapid flow of viscous fluid inside the network, colloidal or microscopic, of the muscle. Hence, on stretching the muscle rapidly by increasing the tension on it, there will be a production of heat under two headings—

(a) The reversible thermodynamic effect given by the formula deduced above,

$$Q = \tfrac{1}{2}(-\alpha)\,T\,(P_1 - P_2)\,(l_1 + l_2)\,;$$

(b) The irreversible effect corresponding to the area OCA in the diagram.

These effects are of the same sign and additive.

When the muscle is unloaded a similar process takes place. If unloaded infinitely slowly, the extension-tension relation follows the curve AO. If unloaded more or less rapidly, it follows one or other of the curves AB′O, AC′O, AD′O. The *maximum* work obtainable from the stretched muscle is given by the area AOa. The work obtained in a rapid shortening corresponds to one or other of the areas AD′a, AC′a, AB′a, the area AB′a corresponding to the more rapid process. Consequently, in shortening rapidly along (say) the curve AC′O work is lost corresponding to the area AC′O, the potential energy of the stretched muscle re-appearing partly as external work equal to the area AC′a and partly as heat equal to the area AOC′. This irreversible transformation of potential energy into heat also is presumably due to the flow of viscous fluid inside the elastic network of the muscle. Thus, on unloading the muscle rapidly there will be two different thermal effects :—

(a) The reversible thermodynamic effect given by the formula deduced above, viz., an absorption of heat equal to

$$\tfrac{1}{2}(-\alpha)\,T\,(P_1 - P_2)\,(l_1 + l_2)\,;$$

(b) The irreversible production of heat corresponding to the area AC′O in the diagram.

These effects are of opposite sign and lead to the complex curves of unloading shown in figs. 1 to 5.

A further interesting case arises in the alternation of loading and unloading at a finite interval, as shown in figs. 2, 3, and 5. Here the relation between tension and extension follows a set of curves more or less similar to those shown in fig. 7. When the muscle is loaded, the extension follows the curve OA; as it remains loaded it stretches at constant tension from A to B, from A to C, or from A to D, according as a shorter or a longer interval is given. If the muscle be unloaded a short time after it is loaded, the complete cycle is represented by the curve OABG; if the interval be longer, by the curve OACF; if still longer, by the curve OADE. Thus, if the interval between loading and unloading be short, work corresponding only to the area OABG is degraded into heat, while if the interval be greater more work is lost, corresponding to the area OADE. In any case, however, some work is lost. Thus,

in addition to the reversible thermodynamic effects corresponding to loading and unloading, there is an irreversible production of heat, corresponding in each cycle to

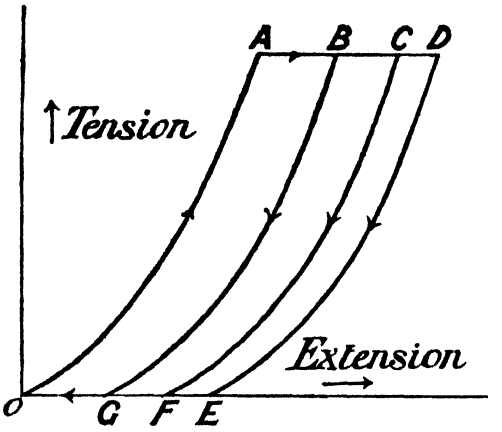


FIG. 7.—Curves relating tension and extension in the case of a complete cycle made up of a rapid process of loading and a rapid process of unloading separated by a finite interval. OA represents the loading curve; BG the unloading curve after a short interval; CF the unloading curve after a longer, and DE after the longest interval. The work degraded into heat irreversibly in the complete cycle, OACF, corresponds to the area OACF, and is clearly greater the greater be the interval between loading and unloading.

[NOTE.—These curves are illustrative only and do not represent an actual case.]

(say) the area OACF, and appearing in fig. 2 as the steady displacement of a curve on which are shown oscillations corresponding to the reversible alternations of heat production and absorption. The same phenomenon is shown in another way in fig. 5. Here the thermal effects of loading and unloading are shown in curves I and II, III, IV, and V, with intervals between them of "infinity" and of 1, 2, and 4 seconds respectively. It can be shown, both experimentally and theoretically, that the total heat produced corresponds fairly accurately to the area of the photographic record, and it is obvious that the areas of curves III, IV, and V are in ascending order of magnitude, corresponding to the increasing interval between loading and unloading. This is a strong incidental verification of the theory sketched above.

A further verification of it is shown by the following observation. In most of the experiments the thread connecting the muscles to the load passed up freely through a narrow dry tube, but in those few in which the tube was filled with vaseline, and the thread passed through the vaseline, it was obvious that the secondary and irreversible rise of temperature following unloading was far smaller than in the other experiments. The reason is simple. It required a definite, if small, force to pull the thread through the vaseline at a finite rate, so that the unloading process really took place very much more slowly, the muscle being held to some extent by the vaseline, and giving up its potential energy gradually in warming the vaseline instead of warming itself. By allowing the muscle to shorten even more slowly, the method might be extended and the irreversible thermal effects eliminated almost completely.

By means of the method of analysis described in another paper (8) it is possible

to determine the rate of heat-production at all moments subject to loading or unloading and to exhibit it in a curve. The details of such an experiment are given below and the results are shown in fig. 8.

Experiment.—Pair of sartorius muscles. Permanent load, 7 grm. Extra load of 50, 100, 150, or 200 grm. put "on" or taken "off." Control curves made by electrical warming with 7, 57, 107, 157, and 207 grm. on; the initial shapes of the five sets of control curves agreed exactly. By means of the control curve the loading and unloading curves were analysed. The unloading curves are complex, owing to the mixture of production and absorption of heat; the analysis of them is difficult, and in such a case the results are necessarily rather indeterminate. The loading curves, however, are easy to analyse, and the following analysis of the thermal effects of loading with 200 grm. is typical. Heat production is given on an arbitrary scale in units per $\frac{1}{4}$ second :—

Time, secs.	0	$\frac{1}{4}$.	$\frac{1}{2}$.	$\frac{3}{4}$.	1.	$1\frac{1}{4}$.	$1\frac{1}{2}$.	$1\frac{3}{4}$.	2.	$2\frac{1}{4}$.	$2\frac{1}{2}$.	$2\frac{3}{4}$.	3.
Heat production .	56	14	8	4	3	2	2	2	2	3	2	2	1

After 3 seconds the heat production continued more or less uniformly at the rate of 1 unit per $\frac{1}{4}$ second for some time. The results are shown in fig. 8. The analysis of the unloading curve shows that there is an absorption of heat, following more or less the type of relation shown in fig. 8, but having superimposed upon it a production of heat starting later and falling more rapidly. The absorption of heat is initially the faster, then becomes the slower, and finally the faster again.

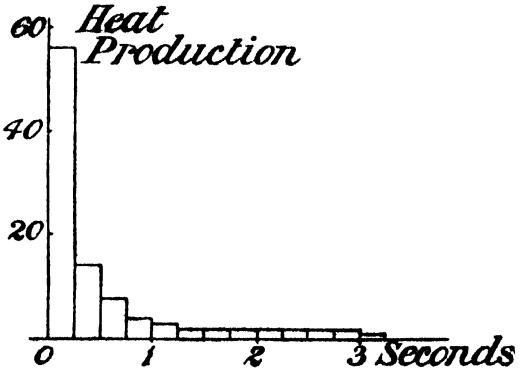


FIG. 8.—Pair of sartorius muscles, permanent load 7 grm., loaded with extra 200 grm. Record analysed and heat production shown in arbitrary units at every $\frac{1}{4}$ sec. following loading. For description of experiment see above.

(4) RESULTS AND DISCUSSION OF MECHANICAL EXPERIMENTS.

A further experimental confirmation of the theories described above may be gained by the use of the inertia system of obtaining the maximum work described by one of us recently (7). A muscle, alive or dead, is connected to the inertia lever by a long thread: the lever is then loaded with a small rider to record the work performed, and a large rider to stretch the muscle. The large rider hangs from a long thread, and, when all is ready, is suddenly lifted off the lever; the stretched muscle immediately proceeds to shorten, and gives out all the work of which it is capable in accelerating the lever; the work done is measured by the distance through

which the small rider is lifted. If the moment of inertia of the lever were infinite, the stretched muscle would shorten infinitely slowly, and give out all its elastic energy as work; the moment of inertia, however, being relatively small, the muscle shortens rapidly, and therefore gives out considerably less work. By loading the muscle with different weights and noting the extension produced, it is possible to construct a curve relating extension to load, such as the curve OA in fig. 6. From this, the potential energy corresponding to any given load can be read off as an area. This potential energy can then be compared with the actual work performed on the inertia lever in an ordinary rapid shortening consequent on release. It is found that the actual work done in a rapid shortening may be very considerably less than the potential energy of the muscle, *i.e.*, than the work which would be done in a very slow shortening; consequently, in a rapid shortening, a large part of the potential energy is wasted, and can only reappear as heat. The following Table gives typical results :—

TABLE I.—Comparison of the work done in the rapid shortening of a passively extended muscle, with the elastic potential energy possessed by the muscle in virtue of its stretched condition.

NOTE.—The action of the muscle pulling on the inertia lever is similar to that of the muscle pulling horizontally on, and so accelerating, a mass, M, suspended freely by a long string. The "equivalent mass," M, of the inertia system was varied in the following experiment, in order to vary the rate at which the muscle shortened; the greater the "equivalent mass," the more slowly will the muscle shorten. We should expect, therefore, to find more work done when the "equivalent mass" is greater, and this proves to be the case.

The potential energy is calculated from the area of the curve relating tension to extension; this and the work done are expressed in grm.-cm.

EXPERIMENT 1.—Pair of Sartorius Muscles of *Rana Temporaria*.

Load on Muscles, grm. weight.	5½.	7.	8½.	11.	17½.	27.	42.	57.	82.
Work done, M = 520 grm. . . .	0·7	1·0	1·3	1·5	2·2	3·2	4·6	6·1	8·8
Work done, M = 910 grm. . . .	0·8	1·0	1·3	1·7	2·5	3·5	5·0	—	—
Work done, M = 1300 grm. . .	1·0	1·3	1·6	2·0	2·9	4·2	5·9	7·6	10·1
Potential energy (M = ∞) . . .	1·0	1·4	1·9	2·5	3·8	6·0	9·4	14·2	20·8

EXPERIMENT 2.—"Equivalent Mass," M = 2900 grm.

Load on Muscles.	8.	10.	13.	17.	26.	40.	63.	86.	123.
Work done	1·3	1·8	2·3	2·9	4·1	5·5	8·4	10·9	14·7
Potential energy . . .	1·3	1·9	2·7	3·6	5·5	9·0	16·2	23·4	37·2

We see from these experiments that *the work done by a muscle in shortening rapidly when suddenly unloaded is less than the potential energy it contains*, and considerably less for large loads; moreover, Experiment 1 shows that, as the rapidity of shortening diminishes (with increasing "equivalent mass"), so the amount of work obtained increases. These facts confirm the theory discussed in connection with fig. 7 above, and have a considerable bearing on the mechanics of muscular contraction, as will be shown later.

There seems, therefore, to be little doubt that the theory employed to explain the thermal effects of loading and unloading, as shown in figs. 1–5, is correct.

(5) Application to Physiological Problems.

The importance of the phenomena described here can be discussed best under two headings: (*a*) in relation to the heat-production of muscles, and (*b*) in relation to the mechanics of muscular contraction.

(a) *Heat-production.*

Only if a muscle be held rigidly isometric will the heat produced in consequence of a stimulus be free from the complicating effects of the thermo-elastic phenomena described above. If the muscle be allowed to shorten, even as little as 1 mm., the effect will be seen. In analysing the time-course of the evolution of heat in the earlier stages of a muscular contraction (3), we have noticed more than once that, in addition to the ordinary thermal effects, the analysis required a small evolution of heat, followed by an equal small absorption of heat, corresponding in time to contraction and relaxation respectively; and it was noticed in all such cases that the muscle had been able, by reason of being tied by a long and somewhat extensible thread, to shorten 1 mm. or 2 mm., instead of being, as in most experiments, held rigidly isometric by direct attachment to the upper end of the thermopile. It is clear, therefore, that, where possible, it is safer and simpler to employ rigidly isometric contractions in all investigations of the heat-production of muscles. It is advisable also, when employing a "tension lever," to record the tension set up in an isometric contraction, to ensure that only the minimal shortening is necessary in order to work the lever,* and that the connections to the lever are as inextensible as possible (*e.g.*, not made of silk thread, which stretches considerably). If such precautions be taken, it is possible to avoid largely, if not entirely, the errors and complications caused by the phenomena described here.

In many experiments, however, and in the very important experiments in which work is actually done by the muscle, it is not possible to employ isometric con-

* In order to avoid any appreciable shortening of the muscle it is advisable to adopt a photographic device giving high magnification of movement for recording the tension developed by a muscle. Such a device was used in making the curves shown in fig. 12, of the paper (3) describing the methods employed in this investigation.

tractions. In all such cases, the actual heat-production observed is the resultant of the thermal effects of mechanical shortening or extension, and of the physiological effects set up by the stimulus. This is still further complicated by the fact that, during relaxation, the part of the potential energy set free on excitation which has not been used up in doing work is irreversibly degraded into heat. The matter can, however, be made rather more intelligible by the consideration of a physical analogy. It will be seen from this that, if a muscle, whether excited or not, be released and allowed to shorten, we may expect to find an absorption of heat, in addition to the absorption of mechanical potential energy transformed into work. If the muscle be subjected to a high initial tension, we may expect the initial absorption of heat to be greater. If the tension in an unexcited muscle carrying a weight be provided by one part (A) of the muscle, while the "active" physiological increase of tension consequent on excitation is provided by another part (B), *e.g.*, if the initial tension be taken by inactive connective tissue fibres, while the development of tension is due to active muscle fibres, then the problem is simple, for it is clear that each part can be considered separately; in this case, it is obvious that, when the whole system shortens, doing work, the usual *physical* thermal consequences of shortening must take place in the part (A) of the system, in addition to the combined physical and physiological effects in the part (B). If, however, (A) and (B) be the same, *i.e.*, if the passive tension of the loaded but unexcited muscle be provided by the same fibres or network as develop the extra tension on excitation, the matter is not so simple, and we must consider the thermal consequences of shortening in the excited muscle as a whole. The correct way of regarding the question then is best made clear by considering the following physical analogy.

A Physical Analogy.

Consider a soap-bubble of radius r, consisting of a film with surface-tension β. The surface energy of the bubble is then $4\pi\beta r^2$, and the maximum work obtainable, by allowing the bubble to contract from radius r_1 to radius r_2, is $4\pi\beta(r_1^2 - r_2^2)$. Thus the bubble is analogous to an unexcited muscle subjected to an initial tension, and is capable similarly of doing work, if allowed, at the expense of its stored potential energy. There is, moreover, a further analogy with the muscle. Surface-tension, in general, diminishes with rise of temperature, and, from this, it can be shown thermodynamically that there is a reversible production of heat when the bubble contracts, and similar absorption of heat when the bubble expands; these thermal effects are analogous to those shown on loading or unloading a muscle.

The free energy, A, of the contraction of the bubble is given by

$$A = 4\pi\beta(r_1^2 - r_2^2).$$

Employing the thermo-dynamic equation

$$Q = T\frac{dA}{dT}$$

we find that the heat *absorbed* is given by

$$Q = 4\pi T (r_1^2 - r_2^2) \frac{d\beta}{dT}.$$

But $d\beta/dT$ is negative for actual films of fluid (8) and may be put constant and equal to $-k$, so that the heat *produced* is given by

$$Q = 4\pi kT (r_1^2 - r_2^2).$$

Consider now the case of a soap-bubble which, by some active process, following what (to make the analogy clearer) we will call "excitation," increases its surface tension from β to β'. Many such processes might be considered, *e.g.* :—

(a) The oxidation of a surface film of some substance, such as oil, which previously lowered the surface tension of the water composing the bubble;

(b) The neutralisation of an electric charge on its surface (in this case the mathematical statement is slightly different from that given here);

(c) The liberation of some chemical bodies, raising the surface tension.

There is little advantage, however, in discussing specific cases, as it is not being suggested here that any one of them represents the processes underlying muscular contraction. It is sufficient for us at present to gain a clear *general* idea of the nature of muscular activity, and to leave the description of specific processes to a day when our knowledge is more adequate. We will proceed, therefore, to consider the general problem, and will make the analogy clearer by employing physiological terms.

(A) The "resting" bubble possesses potential energy: if allowed to "contract" it can do work,

$$A = 4\pi\beta (r_1^2 - r_2^2).$$

(B) The "excited" bubble possesses more potential energy: if allowed to "contract" it can do more work,

$$A' = 4\pi\beta' (r_1^2 - r_2^2),$$

where β' is made greater than β by some process undefined. If not allowed to "contract," but made to respond "isometrically," the pressure inside the bubble rises from $2\beta/r$ to $2\beta'/r$.

(C) If the "resting" bubble be allowed to contract, doing maximum work, there is a reversible thermal effect, leading to a rise of temperature; if it be made to expand, there is a similar fall of temperature. The heat produced in the contraction is

$$4\pi kT (r_1^2 - r_2^2),$$

where k is the temperature coefficient of the surface tension.

(D) If the "excited" bubble be allowed to contract, there are similar reversible thermal effects, the heat produced on the contraction being

$$4\pi k'T (r_1^2 - r_2^2),$$

where k' is the temperature coefficient of the new surface tension.

This heat must be added to any produced by the chemical or physical processes leading to the increase of surface tension.

The analogy with the muscle, therefore, is clear, and we should expect that there would be a reversible thermodynamic evolution of heat equal to

$$\tfrac{1}{2}\alpha T (P_1 - P_2)(l_1 + l_2)$$

when the excited muscle shortens from length l_1 (and tension P_1) to length l_2 (and tension P_2). In this case, however, α, the coefficient of thermal expansion, is that of the excited muscle; it would be difficult to devise a direct means of determining this quantity, but there is no reason to doubt its reality. It is presumably negative, as in the unexcited muscle, so that there is then a reversible absorption of heat on contraction, in addition to the transformation of mechanical potential energy into work.

The thermal phenomena described here have no considerable bearing on results *hitherto* obtained on the heat-production of muscles. Most previous work in which the muscles were allowed to shorten over the junctions must anyhow have been vitiated to an unknown degree by differences of temperature along the muscle's length, and conclusions from it are of doubtful value. Most of the more reliable work on the subject was performed on muscles excited isometrically. In the future, however, it will be necessary, as soon as the simpler problem of the isometric contraction has been properly explored, to consider the case of muscles fulfilling their natural, though more complicated, function of shortening; and in that consideration it will be necessary for the investigator to be alive to the difficulties and complications provided by the purely physical thermal effects consequent on the shortening of an extended elastic body.

(b) *Mechanics.*

In considering the mechanics of muscular contraction, the fact that all the potential energy put into a stretched elastic body can be recovered as work *only* if the shortening be infinitely slow is very important. Consider first the case of a muscle passively stretched. If the muscle be *stretched* from a length l_2 to a length l_1, and if the tension P be required to stretch it to any length, l, *then* P *is greater the more rapidly the stretching is carried out.* Consequently, for a given amount of stretching (*i.e.*, for a given final amount of potential energy) the faster the stretching the greater will be the work required and the more wasteful will be the process. Similarly, if the muscle be allowed to shorten from l_1 to l_2, doing as much work as possible, the tension P exerted at length, l, is *less* the more rapidly the muscle is allowed to shorten; consequently, for a given amount of potential energy available, the more rapidly the muscle shortens the smaller is the amount of external work done and the more wasteful the process. What is true of the elastic properties of the unexcited muscle is true also of those of rubber, and in all probability of those of the excited muscle. In that case one very important conclusion follows: *the more slowly a muscle be allowed to contract (in a single twitch) the more work can it be made to do.* This statement should be clearly understood, especially as it may have

a practical application in our study of the heart and other muscles. It should be noted that the contraction considered is supposed to take place between the same geometrical limits in the rapid and in the slow contractions; in the case of the straight muscle it shortens from length l_1 to length l_2 in either contraction, or in the case of the heart it contracts from volume v_1 to volume v_2 in either contraction; with this provision, however, in the case of a twitch (or beat) excited by a single given stimulus, *the work done will be greater the slower the contraction is allowed to go on.* The gain in efficiency merely by slowing down the process of shortening may be relatively considerable.*

A further important application is to the case of a muscle contracting under a relatively heavy initial load. When an inactive muscle is loaded heavily, it possesses a considerable amount of potential energy; when the muscle is excited more potential energy is developed as the result of the physiological processes following a stimulus: if the muscle be allowed to shorten, both of these forms of potential energy are available presumably for the production of work, and it would seem natural to subtract the potential energy of the inactive stretched muscle from the total work done, in order to determine how much of the work was done by the muscle in virtue of its own physiological activity. The results of this paper show that such a procedure is not correct, and, indeed, if the initial load be high, may lead to very erroneous results. When a muscle is excited, it contracts rapidly, and when the stretched inactive fibres of the muscle are allowed to shorten rapidly we have shown above that there is a considerable degradation of potential energy into heat, only a fraction of it appearing as external work. Thus the work actually produced by the excited muscle by virtue of its own internal activity is greater, and may under heavy initial loads be considerably greater, than the quantity calculated as described above; while the heat produced by the muscle, by virtue of its internal activity, is less than the heat actually observed, because of the transformation of some of the potential energy into heat when the muscle shortens. If a correct calculation be desired of the work and heat liberated by the unaided internal activity of the muscle, it is necessary to measure:

(a) A, the potential energy possessed by the stretched inactive muscle;

(b) W, the maximum work obtainable from a mechanical shortening of the inactive muscle *at the same rate of shortening* as obtained in the active twitch.

Then W, and not A, must be subtracted from the work done in the active twitch, in order to get a fair estimate of the share of the work provided by the internal activity of the muscle; while (A—W), expressed in heat units, must be subtracted from the heat-production observed in order to find what amount of this heat is due to the same internal causes.

* This statement must not be misapplied, nor taken from its context.

(6) The Coefficient of Thermal Expansion of Muscle.

It is interesting to calculate the value of α, the coefficient of thermal expansion, from the formula

$$\text{rise of temperature on loading} = -\frac{\alpha T (P_1 - P_2)(l_1 + l_2)}{(\text{mass})(\text{specific heat})(\text{mechanical equivalent})}.$$

On the right side of the formula we can measure everything directly except α, while our records enable us to determine the rise of temperature. As a matter of fact, it is necessary, in the records, to separate the reversible thermodynamic production of heat from the irreversible "viscous" one. We have not succeeded in doing this by any rigidly accurate method, but a more or less approximate value can be estimated from the records. The result comes out that α is negative (*i.e.*, the muscle *shortens* on warming) and in size between 10^{-5} and 10^{-4}. Why the muscle has a negative temperature coefficient it is difficult to say. Rubber appears to have the same, and of about the same order of size. It would be of interest to repeat the observation on jellies and on other kinds of rubber or elastic colloidal material. It is striking, however, that the value of α (lying between 10^{-5} and 10^{-4}), calculated for muscle from the experiments described here, agrees in magnitude, though not in sign, with the values given in Tables for a number of materials. For example, in metals α usually lies between 10^{-5} and 2×10^{-5}, gutta-percha is given the value 2×10^{-4}, glass about 10^{-5}, and various woods about 5×10^{-5}.

(7) Summary.

1. Photographic records, obtained thermo-electrically, are given of the thermal consequences of stretching a muscle, and of releasing a stretched muscle. When a muscle, alive or dead, is passively *stretched*, heat is liberated in large amount at first, but at a rapidly diminishing rate. When a stretched muscle is *released*, the first effect is an absorption of heat, but this is followed, after a short interval, by a production of heat masking the absorption. In a complete cycle of lengthening and shortening the net result is a production of heat, which is greater the greater be the interval between the two processes.

2. These thermo-elastic phenomena are in no way related to the life or visible structure of the muscle, as they are shown by live and dead muscles alike, and, in a modified degree, by a rubber band.

3. The order of size of the thermo-elastic effect may be gathered from the statement that a load of 150 grm., added to a pair of sartorius muscles of *Rana temporaria*, weighing, say, 150 mgr., would raise their temperature by something of the order of 1 to 3 thousandths of a degree, this being a fifth to a half of the rise produced by a strong twitch. These thermo-elastic effects, therefore, are of a size which makes a knowledge of them essential in experimental work on the energetics of muscular activity.

4. The phenomena depend upon the elastic and thermo-elastic properties of the muscle, and may be credited to the simultaneous action of the following two factors :—

(i) The muscle, like catgut, shortens on being warmed ; conversely, the second law of thermodynamics tells us that it will warm on being stretched, and will cool on being released from a stretched state, both processes being "reversible." This explains the initial effects.

(ii) The muscle, like other elastic colloidal jellies, takes some time to reach an equilibrium length on being subjected to a tension ; consequently, on stretching it, *more* work is done, and, on releasing it, *less* work is obtained, than is accounted for by the potential energy existing in it when extended. The balance in either case is liberated irreversibly as heat in the muscle. This explains the later effects.

5. The initial thermal effect—the reversible one, see 4 (i)—enables us to calculate an approximate value for the coefficient of thermal expansion of frog's muscle, and leads to values lying between -10^{-4} and -10^{-5} ; we may assume, therefore, that the sartorius muscle of *Rana temporaria*, subjected to a constant load, shortens by 1 part in something between 10,000 and 100,000 for every 1° C. rise of temperature. The coefficient is of the opposite sign to that for most materials, which lengthen on heating, but is of the same order of size.

6. Experiments are described in which, by means of an inertia system for the determination of the maximum work, the elastic potential energy of the *passively stretched* unstimulated muscle is transformed into work during an elastic contraction occurring at various rates. It is found that the work done is greater the slower is the contraction, but is always considerably less than the potential energy used up. For an infinitely slow contraction, the work done would become equal to the potential energy. The balance of potential energy is used up in irreversible processes, leading to the secondary thermal changes described in 4 (ii) above.

7. It is suggested that the elastic properties of the muscle, leading to the irreversible transformation of work into heat, are the result of its microscopic, ultra-microscopic, or colloidal structure. The relation between tension and extension, as usually found, *i.e.*, leaving the load on till the muscle has settled down to its full extension, is the elastic characteristic of some network ; the spaces between the parts of the network, however, are filled with a viscous fluid, and, when the shape of the muscle is changed, by pulling or releasing it, the viscous fluid has to find a new position inside the network. If the change be very slow indeed, little energy is lost by internal friction ; if, however, the change be rapid, the loss of energy, which increases with the velocity of the fluid, may become large, and lead to a considerable production of heat. The force exerted by the stretched body in such a case is

employed partly in pushing the viscous fluid into its new position inside the network, and partly in doing external work. Naturally, therefore, the external work is less.

8. It is concluded that in order to avoid these complex thermal changes in investigating the heat-production of muscles, it is advisable, whenever possible, to work with rigidly isometric contractions.

9. It is shown that, when an active muscle is allowed to shorten, we may expect to find the thermal changes due simply to shortening superimposed upon those due to the physiological activity (chemical breakdowns, etc.) of the muscle.

10. When a muscle, excited by a single shock, is allowed to shorten from one fixed length to another (or, in the case of the heart, from one fixed volume to another), we may expect to find the external work done greater the slower is the shortening; this may have a practical application in the study of the heart and other muscles.

11. When a muscle is stretched passively by a load, and then excited, the work done is not equal to the potential energy existing initially in the muscle *plus* the work resulting from the internal physiological activity of the muscle. It is less than this by the amount of the potential energy degraded into heat by the viscous processes associated with its rapid change of form. This is of theoretical importance because, in an investigation of the mechanical efficiency of the muscle, it is necessary to determine the work done by the muscle by virtue of its own internal activity, after allowance for work done at the expense of its initial elastic energy; and it is not fair to the muscle to assume that more than a fraction of this potential energy reappears as work.

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VI.—*The Blood Vascular System of the Tuatara*, Sphenodon punctatus.

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[PLATES 6–8.]

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INTRODUCTION.

It is universally admitted that the Tuatara (*Sphenodon punctatus*) occupies a unique place among living Reptiles. By some authorities it is placed in the order Rhynchocephalia, of which it is the sole living representative; others, indeed, have suggested that it should be included in the order Lacertilia, but even in this case it

* The major part of this work was carried out during my tenure of office as Senior Assistant in the Department of Zoology and Comparative Anatomy at University College, London.

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is regarded as being one of the least specialised forms. It is, then, a primitive member of the class, and is now only to be found on certain islands off the coast of New Zealand, being so near to extinction that it has been placed under protection by the Government of that country. Any additions to our knowledge of the anatomy of this form therefore seem worthy of being placed on record, particularly when, as will be discussed later, they have some bearing upon its relationship with allied forms.

The animal is one of considerable importance, from the comparative point of view, and yet strangely enough no account of its blood vascular system as a whole has ever been given, either in general or in detail, nor is there, so far as I am aware, a satisfactory description of the general anatomy of the heart. Certain points concerning the arterial system have been described, but others have been left untouched, and the same may be said of the venous system. Indeed, in the case of the latter, although much comparative work has been done along certain lines, there is no good general account of the veins in any member of the order Lacertilia. This being so, it is hoped that the following pages, which contain a fairly full account of the blood vascular system in Sphenodon, will help to fill a noticeable gap in our knowledge of the circulatory system in the Reptilia. In conjunction with Prof. DENDY'S account (22) of the intra-cranial vascular system in the same species, it furnishes a more complete account of the blood-vessels than is available even for any of the Lacertilia.

In 1908, Prof. DENDY, F.R.S., suggested to my then fellow-student at King's College, Miss A. W. HILL, B.Sc., that she should work out the vascular system of this important type, and this she proceeded to do. For this purpose, she injected several specimens, and made a number of dissections on the injected and on other uninjected examples. She also made a number of laboratory notes and a series of valuable drawings of her dissections. Before the work was completed, however, Miss HILL left the country, taking with her her notes and drawings. After some years, she found that she would not have an opportunity of completing the work, and Prof. DENDY, in view of the valuable nature and rarity of the material, asked me if I would finish it. This I gladly consented to do, and he placed the original specimens at my disposal. Miss HILL also very generously handed over to me all the notes and drawings she had made, and these, together with the dissections she had left, rendered my task much easier. The drawings have been particularly useful, and I have not hesitated to make full use of them, although in all cases where I have used them I have redrawn them with slight alterations, and this has been indicated by appending thereto both our initials. My own observations were made upon the actual specimens, and the laboratory notes like the drawings served as useful checks.

MATERIAL.

The material upon which these observations are based is a series of well preserved specimens of *Sphenodon punctatus* in the possession of Prof. DENDY, who had used

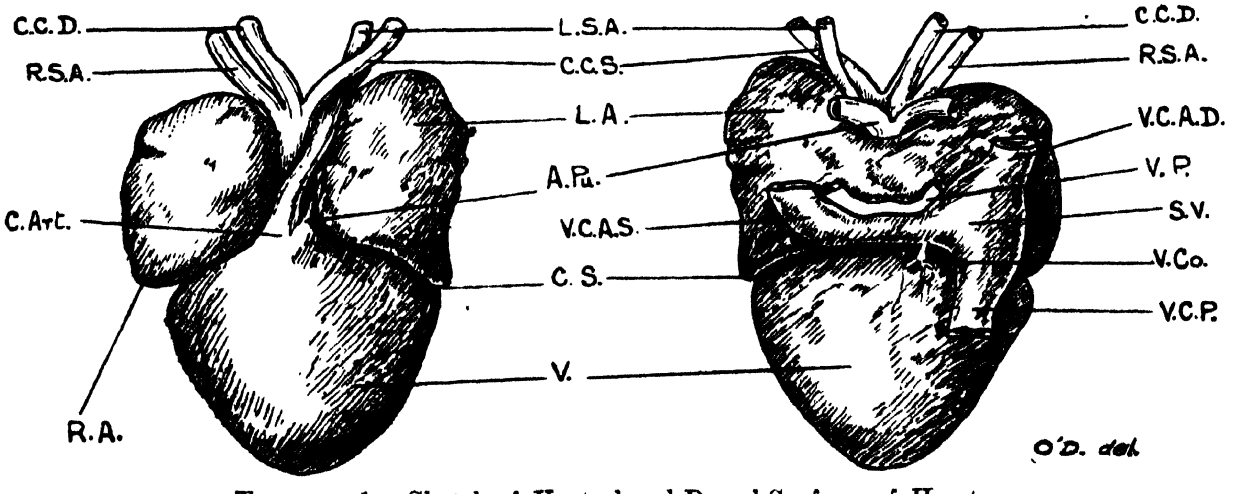
them for his investigations on the intra-cranial vessels (*vide supra*) and on the Pineal apparatus and associated parts of the brain (23). They had been but little touched since they were left, and as they had been carefully preserved, I was enabled to make out practically all the details of the blood vascular system with the exception of one or two unimportant points which are referred to in their appropriate places in the text.

The brains of the injected specimens had been utilised previously, and so, in order to make out accurately the relations of the vessels, particularly the veins of this region, reference was made to series of transverse and longitudinal sections of the heads of late embryos used by HOWES and SWINNERTON in their investigation of the development of the skull (48). They were designated stages R and R–S, and are now in the possession of Prof. DENDY. In this connection I have to thank my former colleague, Dr. K. M. PARKER, of University College, for helping me to make graphical reconstructions of the main vessels of these two stages.

My sincere thanks are due, firstly, to Prof. DENDY for affording me an opportunity of examining such an interesting and valuable species; and, secondly, to Miss A. W. HILL for unreservedly placing in my hands notes and drawings made from dissections which, as I well know, took a great deal of time and exhibited no little skill. Every advantage was taken of this work of Miss HILL, and I am conscious that some of the results set forth below would not have been possible without it.

THE HEART (text-fig. 1).

In outward appearance the heart of *Sphenodon* is typically reptilian; it is not so elongated as in the Ophidia and many of the Lacertilia, nor so broad as in the



TEXT-FIG. 1.—Sketch of Ventral and Dorsal Surfaces of Heart.

A.Pu., arteria pulmonalis; *C.Art.*, conus arteriosus; *C.C.D.*, carotis communis dextra; *C.C.S.*, carotis communis sinistra; *C.S.*, coronary sulcus; *L.A.*, left atrium; *R.S.A.*, right systemic arch; *S.V.*, sinus venosus; *V.*, ventricle; *V.C.A.D.*, vena cava anterior dextra; *V.C.A.S.*, vena cava anterior sinistra; *V.Co.*, vena coronaria; *V.C.P.*, vena cava posterior; *V.P.*, vena pulmonalis.

Crocodilia. It is about 28 mm. long in an average specimen, and when fairly well distended the atria measure about 30 mm. across. It lies, as in other forms, well forward in the pleuro-peritoneal cavity, practically in the median plane and between the lungs.

Sinus Venosus.—The sinus venosus is a well-marked thin-walled sac lying on the dorsal side of the heart just in front of the ventricle; it runs transversely, and the larger part of it is on the right side. It appears to be formed mainly by the confluence of the right anterior caval and the post-caval veins, and their openings are not separated by a semi-lunar ridge, the tuberculum intervenosum, such as we find in the Mammalia. Just to the left of the post-caval vein the coronary vein enters the sinus; it appears as a small but tough cord passing from the sinus across the coronary sulcus on to the wall of the ventricle, as in reptiles generally. To the left of this again, practically in the middle line of the heart, the left anterior caval vein enters the sinus. Outwardly it almost looks as if it were opening into the right atrium, but on slitting the wall of the sinus it will be seen opening into that structure. The aperture leading from the sinus to the atrium is guarded by two distinct valves about 5 mm. long and quite as well developed as in the Amphibia. These sinu-atrial valves lie in the median dorsal wall of the atrium, and run outwards and forwards at an angle of about 75 to the antero-posterior axis of the heart.

Atrium Dextrum.—The right atrium is actually larger than the left, but, as it does not pass so far caudally as the latter, it looks somewhat smaller when seen from the ventral surface. From its antero-dorsal edge near the middle line it gives off a small sac-like diverticulum with thin walls, and in two specimens in which the atria were distended with blood, this showed above the diverging bases of the Carotid arteries. It would appear, therefore, as if it was a normal structure, but its function is not obvious. Two portions of the atrium may be distinguished, the main cavity with thin walls and situated mesially and a postero-lateral auricle (auricula cordis) whose walls are thicker and marked internally by interlacing muscular ridges, the musculi pectinati. The two divisions, however, are not sharply defined and merge into one another more gradually than in the mammal.

Atrium Sinistrum.—The left atrium is on the whole very similar to the right in general structure and appearance. It is slightly smaller in size and the auricle at its postero-lateral corner overlaps the ventricle more than does the right. The two pulmonary veins open into its postero-mesial walls by a common aperture, which, while not guarded by a definite valve, is partly hidden by a fold of the atrial wall which may function as such.

The two atria from the outside seem to form a single sac, but internally they are completely separated by the septum atriorum. The septum, although slightly thinner in its central region, does not exhibit a distinct fossa ovalis such as we find in the Mammalia.

Ventriculus.—The ventricle is a stout-walled sac presenting somewhat the shape of an equilateral triangle when viewed from the ventral surface, and it is about 18 mm. long. It is sharply marked off from the atria by a well-developed coronary sulcus. Its apex is very bluntly rounded, and in one of the specimens examined bore short processes resembling the gubernaculum cordis that BEDDARD has stated to be present generally in the Lacertilia (3). The base of the ventricle is not quite at right angles to the long axis of the heart owing to the fact that the right shoulder is far more rounded off than the left. On the other hand, however, the left auricle overhangs the ventricle more than the right. The dorsal wall of the ventricle is more flattened than the ventral.

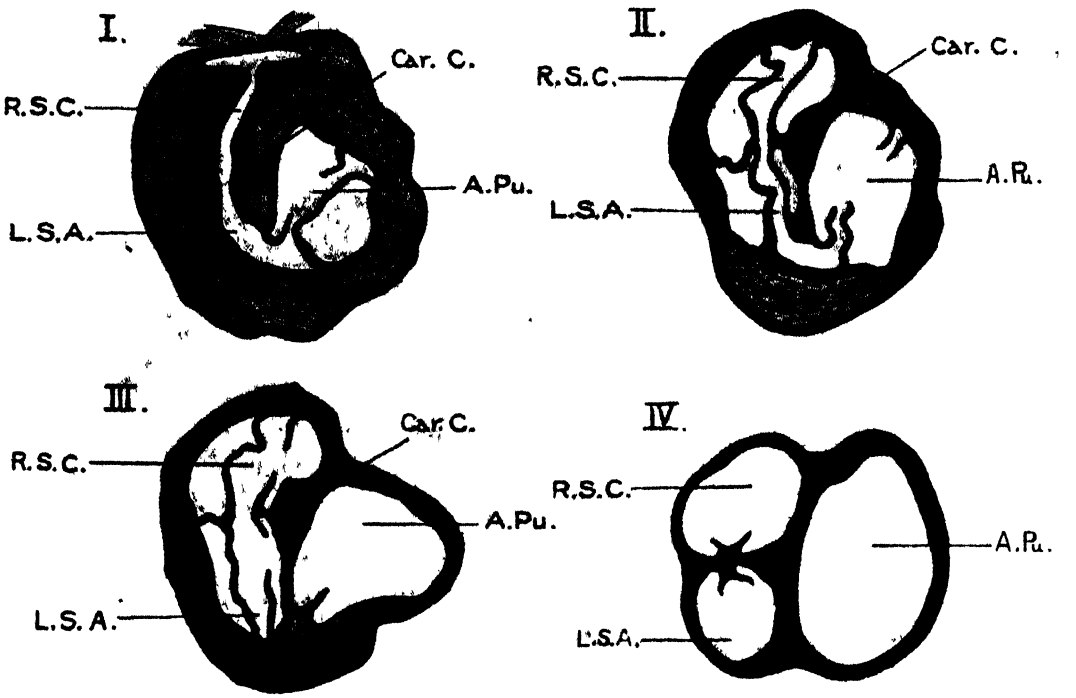
The arterial trunks come off slightly to the right of the middle line from a small anterior projection of the ventricle which, I think, is to be regarded as a remnant of the conus arteriosus for, although it does not bear characteristic conus valves, it nevertheless has valves and constitutes a sort of common trunk from which the arteries arise.

The lumen of the ventricle is quite small owing to the thickness of the wall, which is spongy and composed of an interlacing network of muscular trabeculæ into the interstices of which the blood can penetrate for a considerable distance. The two atria communicate with the ventricle by a common atrio-ventricular aperture situated on the dorsal side of the ventricle at its anterior end. They remain partially separated by an extension of the inter-atrial septum up to the opening which is guarded by a relatively thick flap that separates it from the base of the arterial opening, but does not appear to form a very definite valve. However, the state of preservation of the interior of the heart makes it difficult to speak with certainty on this point.

According to GOODRICH (36A) a small but distinct muscular septum ventriculorum is present in *Sphenodon*. A similar statement is made by GREIL (37), although in neither case is it figured or described in detail. This is a point on which I can make no definite statement as the preservation of the inside of the heart was not good enough to settle the point satisfactorily.

Trunci Arteriosi (text-figs. 1 and 2).—The arterial trunks, as has been noted, come off from a slight projection of the ventricle which is probably to be regarded as a remnant of the conus arteriosus. The three trunks, each guarded by a pair of deep semi-lunar valves, present certain points of interest. Viewed externally there appear to be three vessels from the commencement. These are the left systemic trunk, lying ventrally to the right, a right systemico-carotid trunk (*i.e.*, the vessel from which arise the right systemic arch and the two common carotids), lying dorsally and slightly to the right and the pulmonary arch, lying to the left side somewhat dorsally but just visible at the base of the ventricle from the ventral side. A reconstruction revealed the fact that the internal separation of the trunks was not complete, a point that was afterwards verified by careful dissection. At the base the three vessels stand

in open communication with one another and pass backwards a short distance before their common lumen opens into that of the ventricle, and behind this again lies the atrio-ventricular aperture. This is, I think, the portion to be looked upon as the remnant of the conus arteriosus, and although only short is nevertheless quite distinct, and as it bears at its base one pair of semi-lunar valves obviously cannot be considered as part of the ventricle.



TEXT-FIG. 2.—Outline sketches of four sections through the Main Arterial Trunks, drawn with a Zeiss Meyer camera lucida and stand.

I. At the level where all three arterial trunks are in open communication and posterior to the valves of the right systemico-carotid and left systemic trunks (slide 14, No. 5).

II. At the level where all three trunks are still in communication but the valves in right systemico-carotid and left systemic trunks are fully developed (slide 17, No. 1).

III. At level where main septum completely separates the pulmonary artery but the right systemico-carotid and left systemic are in communication (slide 19, No. 8).

IV. At level where all three trunks are completely separated; at front limit of valves and anterior to the cardiac cartilage (slide 22, No. 10).

A.Pu., arteria pulmonalis; *Car.C.*, cardiac cartilage; *L.S.A.*, left systemic arch; *R.S.C.*, right systemico-carotid.

THE ARTERIAL SYSTEM.

The groundwork of our modern knowledge of the arterial system in the Saurians was laid by CORTI, in 1847, in his detailed account, 'De Systemate Vasorum *Psammosauri Grisei*,' in which he also gives a short account of previous work. This was followed ten years later by a splendid paper by RATHKE (66), dealing with the

aortic roots in general and based upon an examination of 55 different species of Lacertilia and Crocodilia. He did not, however, examine *Sphenodon*. Since this time considerable additions to our comparative knowledge of various parts of the system have been made. In the first place, RATHKE (67) and HOCHSTETTER (44) have described the main gut arteries in many species. Secondly, ZUCKERKANDL (82, 83, and 84) has treated the arteries of the fore and hind limbs in a series of Amniotes, including Lacertilians; and, thirdly, BEDDARD (3, 4, 5, and 7) has investigated the relationship of various arteries in a number of different forms.

As far as *Sphenodon* itself is concerned, it has only been very incompletely studied. VAN BEMMELEN (10), in 1887, wrote a description of the neck region, in which, although mainly concerned with the nerves and their primitive relations to the branchial arches, certain of the neck arteries are accurately described. The main limb arteries were examined by ZUCKERKANDL (*loc. cit.*), and BEDDARD (7) dealt with the main arteries coming from the anterior part of the dorsal aorta. The visceral arteries were first treated, but in a very brief manner, by KLAATSCH (50), and this work was later extended by HOCHSTETTER (44). Finally, DENDY (22), from the same specimens he placed at my disposal, described in detail the intra-cranial arteries, which are therefore omitted here, save that their connections with the extra-cranial vessels are pointed out.

The presence of semilunar valves at the bases of the three arterial trunks and the points of origin of these vessels have already been dealt with. They pass forward together on the ventral side between the median walls of the atria, rotating slowly in a clockwise direction as they do so. Thus, the left systemic trunk starts from the conus ventrally and to the right of the group, and leaves the anterior end of the heart on the left side dorsal to the carotid arch. The pulmonary trunk commences on the left side and leaves dorsally; the right systemico-carotid trunk arises on the right dorsal and finishes on the right ventral side. Thus it will be seen that, while there is a certain amount of twisting, it is not as great as in other Reptiles.

The arterial system is best dealt with by describing the course and distribution of the three arterial arches arising from the three trunks, starting with the anterior pair.

The Carotid Arch (Plate 7, figs. 2 and 3).

The two common carotid arteries take origin together practically in the middle line, from the same point on the right systemic arch just as it commences to bend outwards over the trachea. There is thus no joint trunk or carotis primaria for these two vessels.

According to RATHKE (66, p. 62), two different arrangements are met with in the Lacertilia; in the one the two common carotids come off separately from the right systemic arch, and in the other they come off from a common carotis primaria, which in its turn arises from the right systemic. Even when present, the carotis primaria varies considerably in development, reaching its maximum in the *Varanidæ*, where it

is quite long, and becoming so small in certain groups, including the *Lacertidæ*, that it may readily be overlooked. The two vessels in *Sphenodon* certainly have one common opening into the right systemic arch, but they do not run together until this point, and so it is not possible to speak of a carotis primaria in this form. It is difficult to say whether this approximation to the condition of the *Lacertidæ* is indicative of phylogenetic relationship. The point in itself cannot be pressed too far, as a similar range of differences occurs in the Ophidia; in *Boa*, for example, the two common carotids come off separately, while in *Tropidonotus* a well-developed carotis primaria is present (58).

The two common carotids and their branches in *Sphenodon* pursue almost identical courses on the two sides of the neck and head, save perhaps in some of their smaller unimportant twigs, so that the description given for one side will apply equally well to the other.

The Carotis Communis (RATHKE, CORTI) (Plate 7, figs. 2 and 3, text-figs. 6 and 7).—The common carotid runs sharply outwards and slightly forwards round the trachea to the latero-ventral wall of the œsophagus, where it divides into two branches, the external carotid (B) and the internal carotid (C). Close to its origin it gives off a small vessel (A). RATHKE has noticed three different conditions of the common carotid in the Lacertilia in general: (1) In the *Varanidæ*, where the heart lies a long way back from the head, not only is a carotis primaria developed, but the common carotid runs a good way up the neck before dividing into external and internal branches. A ductus caroticus joining the carotid to the systemic arch is not present. (2) Similarly in the *Chamæleontidæ* no ductus caroticus is present save in *Chamæleon planiceps*, where it is still to be found on each side as a very fine vessel visible only with a hand lens. (3) The third group includes most of the remaining forms where the heart, as a general rule, is not far back from the head, and in these a ductus caroticus is present. The condition in *Sphenodon* is very similar to that in the *Lacertidæ*.

A. *Arteria pericardialis* ('Arterie für das Pericard.,' v. BEMMELEN (10)) is a small vessel going to the pericardium.

B. *Carotis externa* (v. BEMMELEN; Ramus trachealicus, CORTI; 'Kehl-Zungenast des Carotidenbogens,' RATHKE) (Plate 7, fig. 3, text-fig. 7).—The external carotid is a fairly small artery arising from the outer wall of the common carotid, and it runs forward close to the œsophageal wall and gradually outwards, keeping laterad of most of the nerves of the neck. It is a continuation forward of the ventral aorta of the embryo. With the exception of a small thyroid branch (B. I), it remains a single trunk up to the hyoid region. Just after crossing the outer surface of the ceratohyal it is itself crossed ventrally by the hyo-maxillary branch of the twelfth cranial nerve, the largest nerve in this region, and then it immediately divides into two main

branches, the hyomandibular (B. II) and the superficial pterygoid (B. III), which supply the various muscles and tissues of the mental region and the postero-lateral part of the jaws.

According to RATHKE, the external carotid in Lacertilians arises very close to the " Kopfast," *i.e.*, the internal carotid, after the place where it receives the ductus caroticus, but in certain forms, including *Lacerta ocellata* and *L. agilis*, these two points are situated some distance apart. The condition in *Sphenodon* most nearly approaches that found in the *Lacertidæ*.

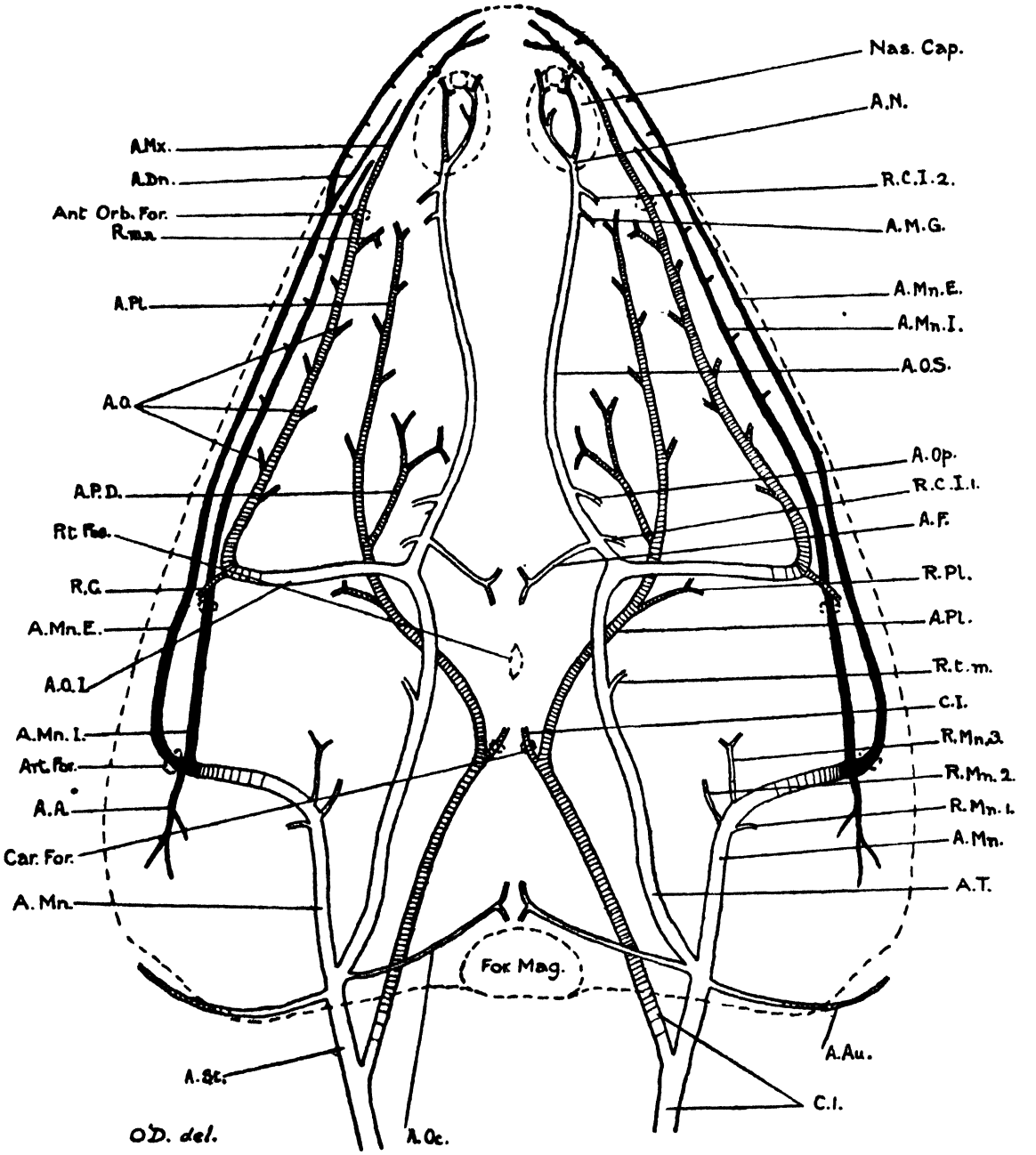
B. I. *Arteria thyreoidea superior* (v. BEMMELEN, 'Thymusdrusen Ast,' RATHKE). —The thyroid artery is a small vessel coming off from the external carotid immediately after the origin of the latter. It runs forwards and inwards, entering the lateral lobe of the thyroid gland.

RATHKE states that in the Lacertilia this branch may sometimes come from the external carotid and sometimes from the common carotid, but he erroneously calls it a thymus branch, as he had previously made the mistake of identifying the thyroid as a thymus gland. VAN BEMMELEN (10) gives two figures of this vessel in *Sphenodon*: in Plate I, fig. 1, it is depicted as arising from the common carotid, which, as far as my observations go, is incorrect; but in Plate I, fig. 6, he shows it correctly coming from the external carotid. The first of these figures, although very good in many ways, somewhat distorts the arteries in order to display the nerves, with which the author was more particularly concerned, and this may account for the inaccuracy.

B. II. *Arteria hyomandibularis* (A. sub-mentalis superficialis, CORTI (text-fig. 3)).—This is the inner and slightly larger of the two branches into which the external carotid divides. It gives off a glossopharyngeal branch (II. α) shortly after its origin, and then runs forwards along the inner border of the hyoideus muscle to its anterior end. Here it passes along the inner border of the end of the anterior cornu of the hyoid and then divides into two main trunks, a submandibular (II. β) and a genioglossal (II. γ).

B. II. α. *Arteria glossopharyngeus.*—The glossopharyngeal artery arises from the base of the hyomandibular and passes dorsal to the hypoglossal nerve to take up a position on its inner side. It accompanies this nerve forward, giving branches to the hyomaxillaris muscle, and on into the genioglossus muscle, to which it also gives branches. It can be followed along the outer ventral border of this muscle almost to its anterior end.

B. II. β. *Arteria sub-mandibularis* (text-fig. 7).—This is a well-marked vessel, situated on the outer ventral surface of the hyomandibularis muscle and running forward on its right to its anterior end at the symphysis menti.



TEXT-FIG. 3.—Diagram of the Main Arteries of the Head, exclusive of the Lower Jaw, viewed from above. Ventral arteries solid, dorsal arteries clear, and intermediate arteries shaded.

A.A., arteria articularis; *A.Au.*, arteria auricularis; *A.Dn.*, arteria dentalis; *A.F.*, arteria frontalis; *A.M.G.*, arteria musculo-glandularis; *A.Mn.*, arteria mandibularis; *A.Mn.E.*, arteria mandibularis externa; *A.Mn.I.*, arteria mandibularis interna; *A.Mx.*, arteria maxillaris; *A.N.*, arteria nasalis; *Ant.Orb.For.*, anterior orbital foramen; *A.O.*, arteriæ orbitales; *A.Oc.*, arteria occipitalis; *A.O.I.*, arteria orbitalis inferior; *A.Op.*, arteria ophthalmica; *A.O.S.*, arteria orbitalis superior; *A.P.D.*, arteria palatina dorsalis; *A.Pl.*, arteria palatina; *Art.For.*, articular foramen; *A.St.*, arteria stapedialis; *A.T.*, arteria temporalis; *Car.For.*, carotid foramen; *C.T.*, carotis interna; *For.Mag.*, foramen magnum; *Nas.Cap.*, nasal capsule; *Pit.Fos.*, pituitary fossa; *R.C.*, ramus coronoideus; *R.C.I.* 1 and 2, rami musculares of the arteria orbitalis superior; *R.m.n.*, ramus membranæ nictitantis; *R.Mn.* 1, 2, and 3, rami musculares of the arteria mandibularis; *R.Pl.*, ramus muscularis of the arteria palatina; *R.t.m.*, ramus temporo-masseteris.

It gives off branches to the adjacent musculature throughout the whole of its course, the largest of which are the following :—

II. β. i. The *Ramus musculo-mandibularis* is a large artery, passing outwards and upwards towards the inner surface of the mandible and dividing into two main branches, which enter the platysma muscle.

β. i. a. A *Ramus ventralis*, that is distributed to the main mass of the posterior part of the platysma muscle.

β. i. b. A *Ramus dorsalis*, that sends twigs into the deeper layers of the platysma and then runs along the inner surface of the mandible.

II. β. ii. The *Ramus muscularis* 1, a small artery that turns inwards and branches in the substance of the hyomaxillary muscle.

II. β. iii and iv. *Rami musculares* 2 and 3 also pass inwards but are distributed to the superficial and deeper layers of the genio-hyoideus muscle.

II. β. v–viii. *Rami musculares* 4–7, are distributed to the anterior portions of the platysma muscle.

II. γ. *Arteria genioglossa.*—The genioglossal artery leaves the main trunk at the level of the larynx and passes inwards and forwards along the side of the genioglossus muscle, to which it gives branches, to end just behind the mandibular symphysis.

B. III. *Arteria pterygoideus superficialis* (Plate 7, fig. 3, text-fig. 7).—The superficial pterygoid artery leaves the external carotid just after this vessel is crossed by the hypoglossal nerve and passes outwards. It gives off small branches to the ventral surface of the internal pterygoid muscle, along which it runs forwards, and at the anterior end of that muscle gives off one or two small branches to its dorsal surface. It is continued forward, branching repeatedly in the strip of subcutaneous tissue that lies between the hyomandibular muscle and the inner aspect of the mandible, and finally ends somewhere in the region of the anterior end of the hyoid (Processus entoglossus of OSAWA (62)).

C. *Carotis interna* (last part of Carotis communis, CORTI ; 'Kopfast des Carotidenbogens,' RATHKE) (Plate 7, fig. 3, text-fig. 3).—The internal carotid artery is a large artery, at least twice the diameter of the external carotid and, from the point of origin of the external carotid, it passes outwards and very slightly forwards over the ventral œsophageal wall. A little way along it bears on its posterior wall a small nodule, the carotid gland, and just beyond this receives the ductus caroticus (C. I). At this point it turns sharply forward, passing along the side of the œosophagus and is now, of course, the continuation forward of the old dorsal aorta of the embryo. Between the carotid gland and the point of entry of the ductus caroticus, the nervus laryngeus superior, a branch of the Vagus, loops around the internal carotid and runs forward up

the neck. According to VAN BEMMELEN (10) the carotid gland communicates with the second thymus gland by a very fine strand of tissue. Unfortunately owing to the condition of the specimens I examined I have not been able to verify this observation.

A fairly large lymphatic vessel appears to enter the internal carotid on its ventral side at the same level as the ductus caroticus. This lymphatic trunk runs along the outer side of the internal carotid and alongside the vagus, passing under the internal jugular vein, and it is dilated just where it enters the carotid. The internal carotid continues forwards immediately below the second thymus and then outside the first thymus, at the anterior end of which it passes below the trunk of the hypoglossal nerve just as this is bending round towards the mental region. The artery then goes on, passing in succession superficially to the cervical sympathic nerve, the glosso-pharyngeal just as this curves outwards on the œsophageal wall, the internal jugular vein, and the vagus nerve. During this part of its course it gives off small twigs to the adjacent neck muscles, *e.g.*, the cucullaris, parieto-mandibularis, longissimus, etc.

Immediately after passing the vagus it gives off a very large trunk, the stapedial (B. II), and itself turns downwards and slightly inwards to run forward first on the lateral wall of the quadrate, then below the auditory capsule and finally on the base of the skull. Beneath the basisphenoid bone it gives off a fair-sized branch, the palatine (B. III), and then enters the cranial wall through the carotid foramen in the basisphenoid. It mounts in the wall of the sella turcica laterad of the hypophysis, to which it gives a small twig, and then in the substance of the cranial wall, which is here membranous, to a point just below the exit of the oculo-motor nerve and then turns inwards, becoming completely intra-cranial, to the latero-ventral wall of the brain. Here on the ventral surface of the crus cerebri it divides into an anterior (B. IV) and a posterior (B. V) branch. The distribution of these branches has already been worked out very carefully and fully by DENDY on the same specimens employed here, and is described in his paper on "The Intracranial Vascular System of Sphenodon" (22) and so need not be repeated.

C. I. *Ductus caroticus.* (Absteigender Schenkel des Carotidenbogens, RATHKE; junction of the Carotid and Aortic Arches, VAN BEMMELEN) (Plate 7, fig. 3, text-figs. 6 and 7).—This is a well-marked vessel of about the same calibre as the internal carotid itself. It runs from the postero-dorsal wall of the carotid, where it turns forwards immediately beyond the carotid gland, to the outer wall of the systemic arch a short distance in front of the ductus Botalli, thus putting the aortic and carotid arches in free communication with one another. Slightly nearer the systemic arch it gives off a moderate-sized branch (I. α), the muscularis cervicis. Some confusion exists in the

nomenclature of this vessel, as it has been termed the ductus Botalli by BEDDARD (3, 7 and 8) in certain lizards, whereas it is more properly called the ductus caroticus, as it was first named by BRANDT (17). This matter, together with the distribution of the two ductus in Reptilia, I have dealt with more fully previously (60), and it is only necessary to call attention to one or two points therein mentioned. It is not found in the *Varanidæ* nor as an open vessel in the *Gekonidæ*. Although present in many other Lacertilians, it is very often reduced in diameter or even a solid strand, a ligametum caroticum, but in none of these is it less specialised than in *Sphenodon*.

The presence of both ductus caroticus and ductus arteriosus (Botalli) is a point of considerable importance. As they are to be found in the embryos of the Amniota, in adult Urodele Amphibia according to BOAS* (12, 13 and 14) and FRITSCH (28), and in the very young frog at the time of metamorphosis, we may justifiably conclude that the presence of both vessels in the higher forms indicates the retention of a fairly primitive condition. Indeed we may say, as has been pointed out elsewhere (60), "In this particular, as far as is known, *Sphenodon* is more primitive than any other living reptile."

C. I. α. *Arteria muscularis cervicis* (VAN BEMMELEN).—The cervico-muscular artery is a vessel running dorsally to supply the muscles in the region of the nape of the neck and the shoulders. According to VAN BEMMELEN it also gives off branches to the thymus glands, but I have not been able to verify this.

C. II. *Arteria stapedialis* (Carotis externa, CORTI; A. facialis, RATHKE) (Plate 7, fig. 2, text-fig. 3).—As has been noted already, the stapedial artery leaves the carotid just after this passes the Vagus nerve. It is a large trunk, considerably larger indeed than the continuation of the internal carotid, and its ramifications cover the whole of the temporal, orbital and a large part of the maxillo-mandibular region. The stapedial runs forward on the median side of the quadrate bone, between it and the cranial wall and then immediately ventrad of the columella auris. Just beyond the columella it gives off a small auricular twig (II. α) and then divides into two large almost equi-sized trunks, the temporal (II. β) and the mandibular (II. γ). Before it divides, the stapedial artery, which was ventral to the vena capitis lateralis, passes laterally around this vein so that the bifurcation takes place dorso-lateral to it.

C. II. α. *Arteria auricularis* (CORTI).—The auricular artery is a small vessel

* In as far as this statement refers to *Triton* and *Salamandra*, I have been able to confirm it by dissection.

which follows the chorda tympani along the posterior wall of the tympanic cavity. It passes along the posterior border of the second epibranchial, giving off in the tympanic region a small posterior tympanic artery which perhaps anastomoses with the external carotid artery.

C. II. β. *Arteria temporalis* (A. facialis, RATHKE) (Plate 7, fig. 2, text-fig. 3).—The temporal artery is slightly the larger of the two branches into which the stapedial divides. After giving off a small occipital branch (β. i) it passes upwards and forwards along the upper border of the cranium between the fascia of the longissimus muscle, to curve forward over the external pterygoid muscle. It gives off a small branch to the temporo-masseter muscle (β. ii) and at the anterior border of the pterygoid it splits into two main trunks, the supra-orbital (β. iii) and the infra-orbital (β. iv) arteries.

II. β. i. *Arteria occipitalis.*—The occipital artery is a small vessel running upwards in the longissimus muscle, to which it gives branches, on the side of the parietal bone. It divides into anterior and posterior branches.

II. β. ii. *Ramus temporo-masseteris.*—This is a small twig, supplying the similarly named muscle.

II. β. iii. *Arteria orbitalis superior* (RATHKE; A. orbito nasalis, GAUPP) (Plate 7, fig. 2, text-fig. 3).—The supra-orbital artery is a large one, continuing more or less in the line of the temporal. At the posterior end of the orbit it gives off three branches: one (iii. a) to the region of the frontal bone, another (iii. b) to the retractor muscle of the eye, and the third (iii. c), which runs outwards to the back of the eyeball, and is distributed to its muscles. The main trunk then continues forward in the dorsal wall of the orbit, and then penetrates the cartilaginous inter-nasal septum. In this it runs below the ophthalmic nerve, and leaves the septum again to re-enter the orbit at its anterior end. Here two branches are given off, one (iii. d) to the glands and muscles, and another (iii. e) to the inferior oblique muscle, and the artery goes on as the nasal (iii. f) into the olfactory capsule.

II. β. iii. a. *Arteria frontalis.*—The frontal artery is a small branch arising just behind the orbit. It runs upwards to the frontal bone, where it divides into an anterior and a posterior branch, supplying the superficial tissue.

II. β. iii. b. *Ramus muscularis* 1.—A fairly small artery, arising near the frontal, runs to the proximal part of the retractor bulbi muscle of the eye.

II. β. iii. c. *Arteria ophthalmica* (Plate 8, fig. 10).—The ophthalmic is a somewhat larger artery, running downwards on to the back

of the eyeball. It is distributed to the muscles of the eye as follows :—

c. 1. A branch to the posterior rectus.
c. 2. A branch to the anterior part of the retractor bulbi.
c. 3. A branch to the superior rectus.
c. 4. A branch to the back of the eye.
c. 5. A branch to the anterior rectus.
c. 6. A branch to the inferior rectus.

II. β. iii. d. *Arteria musculo-glandularis.*—This runs downwards, giving off a backwardly directed twig to the sclerotic coat of the eyeball. Continuing, it passes through the superior oblique muscle, supplying it with a twig, and on into the Harderian gland, in which it breaks up.

II. β. iii. e. *Ramus muscularis* 2 runs straight downward, and is distributed to the inferior oblique muscle.

II. β iii. f. *Arteria nasalis.*—The remaining part of the supra-orbital may be termed the nasal artery, since it enters the olfactory capsule and is distributed mainly to the olfactory organ, although its terminal branches end in the subcutaneous tissue of the snout. On entering the capsule it divides into two main twigs, the superior and inferior nasal arteries. These run in the floor and roof of the nasal cavity respectively, supplying the mucous membrane and the end of the snout, as indicated above.

II. β. iv. *Arteria orbitalis inferior* (A. dentalis superior, RATHKE) (Plate 7, fig. 2, text-fig. 3).—The infra-orbital artery leaves the temporal at the postero-dorsal corner of the orbit, turning sharply downwards in its hinder membranous wall. In then runs on in the floor of the orbit, accompanying the infra-orbital branch of the trigeminal nerve. In this part of its course it gives off the coronoid (β. iv. a) branch and others to the surrounding tissues (β. iv. b) and nictitating membrane (β. iv. c). At the front end of the orbit it enters the maxilla through the anterior orbital foramen, and so becomes the maxillary artery (β. iv. d).

II. β. iv. a. *Ramus coronoideus.*—This vessel is given off from the infra-orbital just as it turns to run forward in the floor of the orbit. It breaks up into small branches, distributed to the tissue in the coronoid region.

II. β. iv. b. *Arteriæ orbitales.*—While passing through the orbit, the infra-orbital artery gives off small vessels to the adjacent tissue.

II. β. iv. c. *Ramus membranæ nictitantis.*—At the anterior end of the

orbit the infra-orbital artery sends a branch upwards, to supply the nictitating membrane.

II. β. iv. d. *Arteria maxillaris* (RATHKE) (text-fig. 3).—The maxillary artery is really the continuation of the infra-orbital, but deserving of a special name, since it enters the substance of the maxilla by the anterior orbital foramen. It runs forward in the jaw for some way, and finally leaves it, by a foramen at the anterior end of the maxilla, just beneath the external naris, to terminate in the subcutaneous tissues of the snout. While running in the maxilla it gives off four or five small branches, which pass outwards through tiny foramina to the subcutaneous tissues of the upper lip.

C. II. γ. *Arteria mandibularis* (RATHKE) (Plate 7, fig. 2, text-fig. 3).—The mandibular artery passes forwards from the stapedialis along the cranial wall until it reaches a point just below the roots of the trigeminal nerve, when it turns outwards and passes between the internal pterygoid and temporo-masseter muscles, accompanying the mandibular branch of the fifth nerve. Small branches are given off to the temporo-masseter muscle (γ. i and γ. iii) and the internal pterygoid muscle (γ. ii). At the level of the mandible the mandibular artery divides into an internal (γ. iv) and an external branch (γ. v).

II. γ. i. *Ramus muscularis* 1.—A small artery leaves the main trunk just before it reaches the level of the roots of the fifth nerve. It passes outwards, and is distributed to the temporo-masseter muscle in that region.

II. γ. ii. *Ramus muscularis* 2.—A small branch is given off immediately before the mandibular takes up its position alongside the corresponding branch of the fifth nerve. This supplies the internal pterygoid muscle.

II. γ. iii. *Ramus muscularis* 3.—Another branch, arising shortly after the former, runs forward to the anterior part of the temporo-masseter muscle.

II. γ. iv. *Arteria mandibularis interna.*—The inner fork of the mandibular artery passes forward along the inner aspect of the mandible to the coronoid region. Here it enters the bone through a foramen on the inner side of the coronoid bone and continues forward, first in the bone itself, but soon in the deep Meckelian groove. Several twigs leave it during its course, and pass outward to the subcutaneous tissue of the lower jaw.

II. γ. v. *Arteria mandibularis externa* (Plate 7, fig. 2, text-fig. 3).—After parting from the foregoing, this artery gives off a small articular branch (v. a), and then passes through the mandible, *vid* the articular foramen, to the outer aspect of the jaw, where it turns sharply

forwards. In the dentary region it gives off a small dentary branch (v. b), and then goes on to supply the remaining part of the lower jaw up to the symphysis, and also sends twigs to the subcutaneous tissue of the lower lip.

II. γ. v. a. *Arteria articularis* is a small vessel running backwards to the tissues in the articular region.

II. γ. v. b. *Arteria dentalis* is a small branch from the external mandibular that enters the substance of the mandible in the dentary region. It runs forward below the teeth, to which it sends shoots, and it also gives off a few twigs, which pass through the bone to the outside.

C. III. *Arteria palatina* (text-fig. 3).—As has been indicated above, the palatine artery arises from the internal carotid beneath the basisphenoid bone just before the latter artery enters the carotid foramen. At first the palatine runs near the middle line, accompanying the third cranial nerve, and some distance along gives off a muscle branch (III. α), by which time it has turned slightly outwards. At the level of the sub-orbital foramen, a dorsal palatine branch (III. β) is given off. The main trunk runs forward beneath the palatine bone, giving off numerous branches to the sub-mucosa of the roof of the mouth, and finally breaks up in the sub-nasal region.

C. III. α. *Ramus muscularis.*—This branch of the palatine artery passes outwards and forwards, to be distributed to the posterior wall of the orbit, and supplying a well-marked twig to the retractor bulbi muscle.

C. III. β. *Arteria palatina dorsalis.*—This takes a course forward dorsal to the palatine bone, and breaks up into a number of vessels, supplying the tissue in the vicinity.

C. IV. *Ramus cranialis* of the internal carotid.

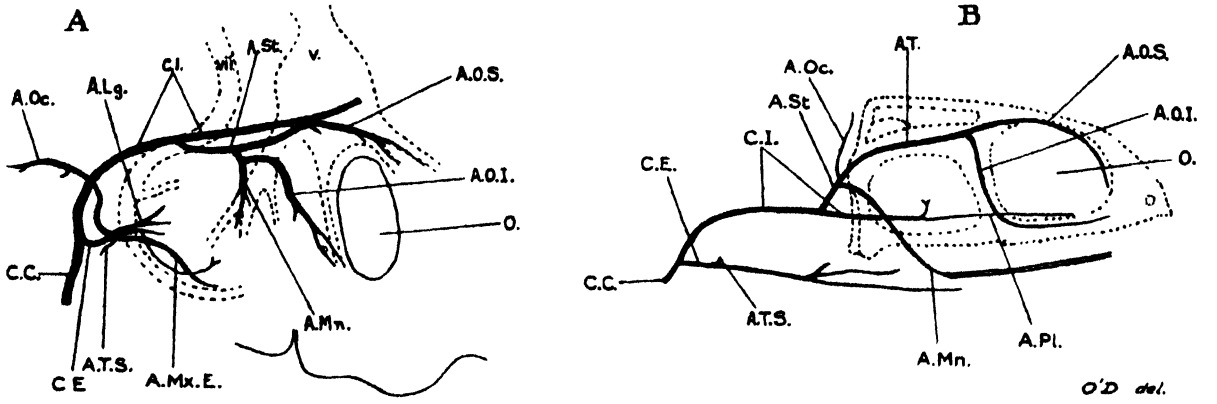
C. V. *Ramus caudalis* of the internal carotid.

These two branches forming the termination of the internal carotid artery and their intra-cranial distribution have already been fully described by DENDY (22), and are only mentioned here for the sake of completeness.

The Distribution of the Carotid Arteries (text-fig. 4).

It seems advisable at this point to call attention to the difference in the territories supplied by the internal and external carotid arteries in *Sphenodon* and the Mammalia respectively. In the mammal, the internal carotid is usually solely intra-cranial in its distribution, while all the remaining parts of the face and jaws are supplied by branches of the external carotid. The condition in *Sphenodon* is very different, however, for here the internal carotid serves not only the brain, but also the whole of the palate, face, and jaws, while the external carotid is relatively a much smaller and

less important vessel, and is concerned mainly with the musculature of the lower jaw. At first sight this striking dissimilarity appears to indicate a fundamental difference between the two conditions, but the reason for it becomes clear in the light of TANDLER'S account (75) of the development of the carotid arteries in mammals. In the mammalian embryo we find the vessels arranged very much as in the reptile; indeed, it is interesting to notice that the arrangement in the 14 mm. human embryo according to EVANS (25) is essentially similar to that in *Sphenodon* and also to that in *Iguana tuberculata* according to RATHKE (67). The actual spatial relations are not the same, of course, owing to the very different proportions of the parts of the



TEXT-FIG. 4.—Diagrams for the comparison of the Internal and External Carotids of the Human Embryo and in *Sphenodon*.

A. Graphic reconstruction of the head arteries in a human embryo measuring 14 mm. Adapted from EVANS (25). B. Diagrammatic representation of the carotids in the adult *Sphenodon*. Approximate position of the skull indicated by dotted lines.

A.Lg., arteria lingualis; *A.Mn.*, arteria mandibularis; *A.Mx.E.*, arteria maxillaris externa; *A.O.C.*, arteria occipitalis; *A.O.I.*, arteria orbitalis inferior; *A.O.S.*, arteria orbitalis superior; *A.Pl.*, arteria palatina; *A.St.*, arteria stapedialis; *A.T.S.*, arteria thyreoidea superior; *C.C.*, carotis communis; *C.E.*, carotis externa; *C.I.*, carotis interna; *O.*, orbit; v and vii, primordia of the fifth and seventh cranial nerves.

head involved, the eye in *Sphenodon* being well in front of the brain, and the brain in the mammalian embryo being relatively larger. The most noticeable points of difference are that in *Sphenodon* the mandibularis artery arises nearer to the origin of the stapedialis (a change perhaps necessitated by the anterior position of the eye). The internal carotid gives off a palatine artery just before entering the skull, and the occipital artery is a branch of the temporal and not of the external carotid (this last difference, however, is not shown by *Varanus griseus*). Although the details of the branches of the external carotid then vary somewhat, the region supplied is essentially the same in each case.

In the mammalia in general an anastomosis is formed during the course of development between the stapedial artery and a branch of the external carotid, and with the later disappearance of the stapedial artery all its branches become transferred to the external carotid. Hence the difference between the regions supplied by the vessels in *Sphenodon* and mammals. The arteria stapedialis appears to persist in

certain mammals, but even when it does, owing to the anastomosis, it gives up to the external carotid the mandibular artery as in the rat (TANDLER, 75), and in addition the infra-orbital artery in the case of the bat (GROSSER, 38).

The Systemic Arch (Plate 6, fig. 1, text-fig. 6).

As mentioned previously, HOCHSTETTER (44) dealt with *Sphenodon* in the course of a comparative account of the arteries of the alimentary canal. It is noteworthy that he considers the relation of these vessels in *Sphenodon* to be primitive and one from which other Lacertilian arrangements can be derived. BEDDARD (7) has furnished an account of the origin of the vessels coming from the systemic arch and the anterior part of the aorta. On the whole, the present work confirms that of these two writers, and also that of ZUCKERKANDL (82, 83, and 84) on the limb arteries. In spite of these works, no complete account of the systemic and aortic branches has yet been given.

The two systemic arches come off separately from the ventricle. The left systemic arch arises as the right ventral vessel of the three coming from the ventricle and it passes in a slight spiral twist over to the left, becoming more dorsal as it does so. The right systemico-carotid is slightly the largest and the most dorsal of the three trunks; it passes slightly to the left and becomes more ventral. As has been pointed out before, it gives off the carotids at the level of the anterior end of the atria, after which it becomes the systemic arch. From this point the courses of the two systemic arches are the same. Each vessel passes laterally and slightly forwards out on to the wall of the œsophagus; at the side of this it gives off a short branch, the anterior œsophageal (A), and exhibits a slight swelling of its wall, the œsophageal gland (VAN BEMMELEN, 10). Here the vessel turns downwards and inwards on to the dorsal wall of the œsophagus. It almost immediately receives on its anterior wall the ductus caroticus, and a short way beyond that the ductus arteriosus (ductus Botalli). The ductus arteriosus is a small vessel connecting the pulmonary and systemic arches. The systemic arches pass backwards and inwards and finally unite in the middle line to form the dorsal aorta. Dorsally to this junction the two sub-clavian arteries (B) are given off. Close examination shows, as BEDDARD (7) has pointed out, that the two arteries actually arise from the right systemic, but just at the point where it joins the left. In *Chamæleon*, according to MACKAY (54), the two sub-clavians arise well up on the right systemic arch. From its point of origin the dorsal aorta runs backwards in quite a normal way, giving off a number of branches (C–N). At the posterior end of the cœlom it passes on into the hæmal canal and as the caudal artery continues right down the tail.

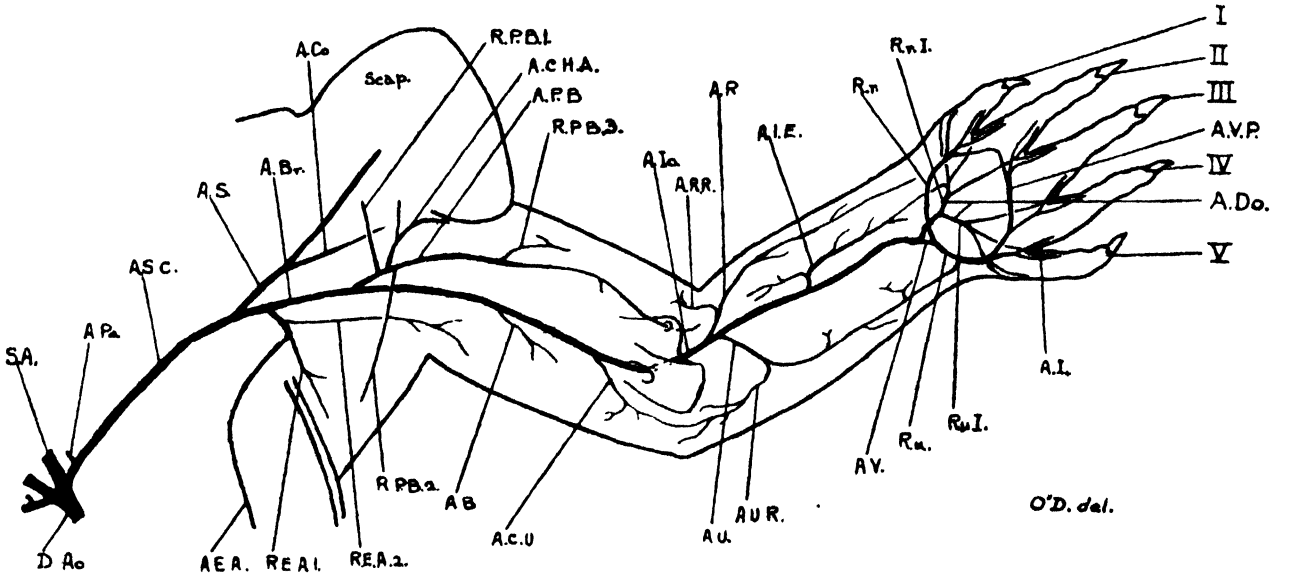
A. *Arteria œsophagea anterior.*—This is a small vessel running forwards up the side of the œsophagus from the systemic arch.

B. *Arteria subclavia* (CORTI) (text-fig. 6).—The origin of the sub-clavian artery has been described above. It passes outwards, giving off three branches

(B. I–III) and enters the arm, where it is termed the brachial artery. In its course along the arm to the entepicondylar foramen it gives off an arteria profunda brachii (B. IV) and other branches (B. V and VI). After passing through the foramen it issues into the muscles of the forearm, where it is termed the arteria interossea, and on its way to the carpus gives off branches (B. VII–X). It finally splits into two terminal branches (B. XI and XII) on the extensor surface of the metatarsals of the fore foot.

B. I. *Arteria parietalis.*—The first vessel is given off by the sub-clavian close to its origin, and is a parietal artery similar to those coming off from the aorta.

B. II. *Arteria scapularis* (Corti).—This is a fairly large vessel going to the shoulder girdle. Its main stem forms a supra-scapular trunk, but it also



Text-fig. 5.—Diagram of the Arteries of the Left Fore-limb from the Flexor Side.

A.B, arteria bicipitalis; *A.Br.*, arteria brachialis; *A.C.H.A.*, arteria circumflexa humeri anterior; *A.Co.*, arteria coracoidea; *A.C.U.*, arteria collateralis ulnaris; *A.Do.*, arcus dorsalis; *A.E.A.*, arteria epigastrica anterior; *A.I.*, arteriæ interdigitales; *A.I.E.*, arteria interossea externa; *A.Io.*, arteria interossea; *A.Pa.*, arteria parietalis; *A.P.B.*, arteria profunda brachii; *A.R.*, arteria radialis; *A.R.R.*, arteria radialis recurrens; *A.S.*, arteria scapularis; *A.S.C.*, arteria subclavia; *A.U.*, arteria ulnaris; *A.U.R.*, arteria ulnaris recurrens; *A.V.*, arteria volaris; *A.V.P.*, arcus volaris profunda; *D.Ao.*, dorsal aorta; *R.E.A.* 1 and 2, rami musculares of arteria epigastrica anterior; *R.P.B.* 1, 2 and 3, rami musculares of arteria profunda brachii; *R.r.*, ramus radialis of arteria volaris; *R.r.* 1, ramus radialis of arcus dorsalis; *R.u.*, ramus ulnaris of arteria volaris; *R.u.* 1, ramus ulnaris of arcus dorsalis; *S.A.*, systemic arch; *Scap.*, scapula; I, hallux; II—V, digits.

gives off twigs to the neighbouring muscles and also a coracoid artery (II. α).

B. II. α. *Arteria coracoidea.*—This is distributed to the musculature in the coracoid region.

B. III. *Arteria epigastrica anterior* (A. mammaria externa, Corti) (text-figs. 5 and 6).—The anterior epigastric artery arises in the axillary region, and

after giving off two twigs (B. III. α and β) passes ventrally along the second rib. It then turns caudally a little to one side of the mid-ventral line, and runs backwards towards the posterior epigastric and the two probably anastomose.

B. III. α. *Ramus muscularis* 1.—A small muscular branch of the epigastric runs to the proximal ends of some of the muscles on the posterior side of the brachium.

B. III. β. *Ramus muscularis* 2.—This is also a small vessel going to the inner sheet of the ventral region of the latissimus dorsi.

The main vessel now becomes the *Arteria brachialis* (text-figs. 5 and 6).

B. IV. *Arteria profunda brachii* (CORTI, ZUCKERKANDL (text-fig. 5)).—The distribution of this artery is fairly typical, the trunk and its branches supply the triceps, supra-coracoideus, coraco-brachialis, and the humeral insertion of the pectoralis, following the nomenclature of OSAWA (62). It gives off branches (B. IV. α, β, and γ) at the proximal end, and another as it passes down (B. IV. δ). Finally, it comes to lie beside the nervus radialis, and may be termed the collateralis radialis (ZUCKERKANDL). It passes with the radialis nerve through the ectepicondylar foramen.

B. IV. α. *Ramus muscularis* 1.—This is a small branch going to the coraco-brachialis muscle.

B. IV. β. *Arteria circumflexa humeri anterior* (CORTI ?).—This vessel passes around the head of the humerus and has approximately the same distribution as the anterior circumflex of CORTI, although it arises from the profunda brachii and not from the brachial artery.

B. IV. γ. *Ramus muscularis* 2.—A small branch runs backwards to the ventral part of the latissimus dorsi muscle.

B. IV. δ. *Ramus muscularis* 3.—A fairly small branch supplies the coraco-brachialis muscle.

B. V. *Arteria bicipitalis* (ZUCKERKANDL) (Plate 8, fig. 8, text-fig. 5).—A vessel arises about half way to the foramen and mainly serves the biceps.

B. VI. *Arteria collateralis ulnaris* (CORTI, ZUCKERKANDL) (text-fig. 5).—A smallish artery arises from the brachial just above the entepicondylar foramen, and runs close to the ulnaris branch of the nervus medianus. It apparently anastomoses with the recurrent ulnar artery.

The main vessel after passing through the foramen may now be termed the *Arteria interossea* (Plate 8, fig. 9, text-fig. 5).

B. VII. *Arteria radialis* (CORTI, ZUCKERKANDL) (text-fig. 5).—The radial artery arises from the main trunk, shortly after it has passed through the entepicondylar foramen and shortly below this point it gives off a branch (VII. α).

The greater part of its course lies in the sulcus radialis, but on nearing the carpus it becomes more superficial.

B. VII. *α. Arteria radialis recurrens* (CORTI, ZUCKERKANDL).—This runs backwards and supplies the muscles in the neighbourhood of the elbow.

B. VIII. *Arteria ulnaris* (CORTI, ZUCKERKANDL) (Plate 8, fig. 9, text-fig. 5).—The ulnar artery arises a little beyond and is more deeply situated than the radial, and after giving off a branch (VIII. *α*) runs near the ulnar nerve to the wrist, where it splits into two. The smaller dorsal vessel passes to the dorsal surface of the hand and the ventral forms a fairly typical *Arcus volaris profundis* in conjunction with a branch from the interosseus (*q.v.*).

B. VIII. *α. Arteria ulnaris recurrens.*—A twig passes backwards supplying the elbow muscles and anastomosing with the collateralis ulnaris artery (B. VI).

B. IX. *Arteria interossea externa* (ZUCKERKANDL) (Plate 8, fig. 8, text-fig. 5).—Rather nearer the wrist than the elbow the interosseus artery gives off a short branch, the external interosseus, which divides into proximal and distal branches that lie close to the dorsal branch of the nervus radialis. The descending branch anastomoses with the arcus dorsalis.

B. X. *Arteria volaris* (Plate 8, fig. 9, text-fig. 5).—When the interosseus artery reaches the ends of the radius and ulna it dives down on to the flexor surface of the hand and under the intermedium gives off a short volar artery. This, together with a terminal branch of the ulnar artery, gives rise to the deep volar arch which commences as two vessels, a radial (B. X. *α*) and an ulnar (B. X. *β*).

B. X. *α. Ramus radialis* (R. medianus, ZUCKERKANDL).—This passes outwards and sends a twig to the outside of the pollex, and one to split over the space between the first and second digits.

B. X. *β. Ramus ulnaris* (R. lateralis, ZUCKERKANDL).—The ulnar branch of the volar arch runs outwards, giving a twig to the outside of the fifth digit and three others. These split over the spaces between digits five and four, four and three, and three and two respectively. From the last of these branches an anastomosis runs to the last branch of the ramus radialis so forming a complete arterial ring in the palm.

The arteria interossea, after giving off the volaris as described above, returns through the carpus just in front of the os intermedium on to the extensor surface of the hand, and here breaks into the two branches (B. XI and XII) which constitute the *Arcus dorsalis.* This peculiar course of the interosseus from the extensor surface down between the ends of the radius and ulna on to the flexor side and then back again through the carpus is evidently primitive, since it is met with in the Amphibia.

B. XI. *Ramus radialis* (ZUCKERKANDL).—The radial branch of the arcus dorsalis receives the anastomosing twig of the external interosseus, and gives off branches to the outside of the pollex and other interdigital twigs to split over the spaces between digits one and two and digits three and four.

B. XII. *Ramus ulnaris* (ZUCKERKANDL).—The ulnar branch of the arcus dorsalis sends a twig to the outside of the fifth digit, and interdigital arteries to the space between five and four and between four and three.

C. *Arteriæ parietales* (text-fig. 6).—From the point of union of the two systemic arteries to form the dorsal aorta, paired parietal arteries come off from this common trunk. Excluding the pair given off from the sub-clavian arteries, seventeen or more usually eighteen pairs can be found before the point of origin of the iliac arteries.

D. *Arteria œsophagea* (HOCHSTETTER).—A small œsophageal artery arises just behind the third parietals.

E. *Arteria gastrica* (HOCHSTETTER) (text-fig. 6).—The gastric artery leaves the dorsal aorta about the level of the fifth pair of parietals and runs to the anterior end of the stomach.

F. *Arteria cœliaca* (HOCHSTETTER; Superior mesenteric, BEDDARD) (text-fig. 6).—The cœliac artery is a large vessel coming from the dorsal aorta near the tenth parietal artery. It is largely concerned with the supply of the stomach and liver, but also serves various other organs by means of a series of branches (F. I–VII), and continues as a large vessel supplying the posterior region of the stomach.

F. I. *Arteria lienalis minor.*—A small artery goes to the spleen.

F. II. *Arteria lienalis major.*—This is the main supply of the spleen.

F. III. *Arteria pancreatica major.*—This is the main vessel going to the pancreas, and shortly after its origin it gives off a small twig to the spleen.

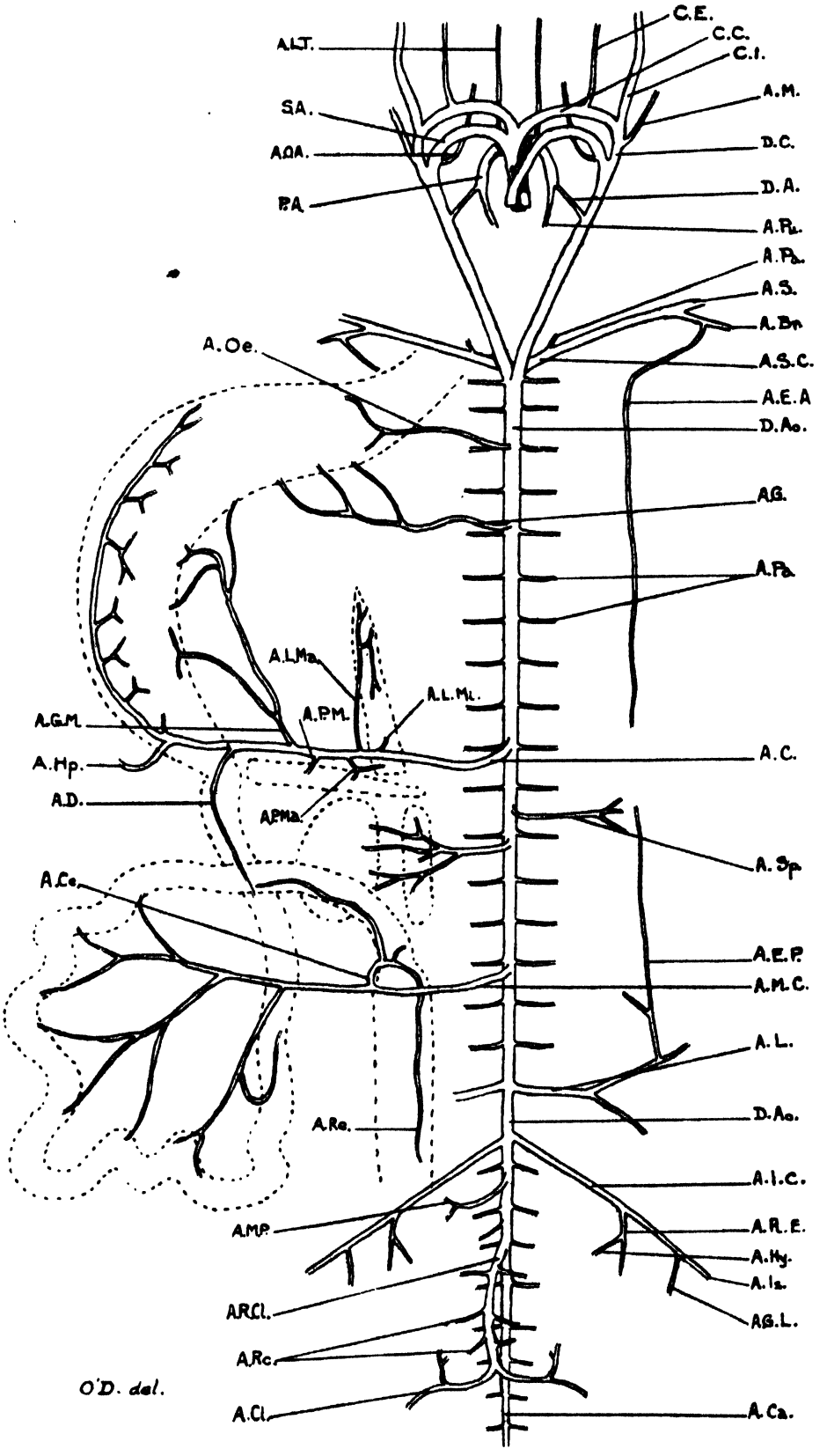
F. IV. *Arteria pancreatica minor.*—A second small twig also goes to the pancreas.

F. V. *Arteria gastrica medialis* (text-fig. 6).—This is a fairly large branch that almost immediately divides into two supplying the mid-region of the stomach. In some specimens the two branches appear to come off separately but close together from the main trunk.

F. VI. *Arteria duodenalis* (A. duodenalis superior, KLAATSCH).—The duodenal artery supplies the proximal part of the intestine.

F. VII. *Arteria hepatica.*—The hepatic artery leaves the main vessel just before it runs on to the stomach wall and enters the liver more or less ventrally.

G. *Arteria spermatica* (text-fig. 6).—This vessel runs to the supra-renal body to which it gives a number of branches, and another set of branches run on to the testis. The left arises a short distance in front of the right. Unfortunately



A.Br., arteria brachialis; *A.C.*, arteria cœliaca; *A.Ca.*, arteria caudalis; *A.Cc.*, arteria cœcalis; *A.Cl.*, arteria cloacalis; *A.D.*, arteria duodenalis; *A.E.A.*, arteria epigastrica arteria; *A.E.P.*, arteria epigastrica posterior; *A.G.*, arteria gastrica; *A.Gl.*, arteria glutea; *A.G.M.*, arteria gastrica media; *A.Hp.*, arteria hepatica; *A.Hy.*, arteria hypogastrica; *A.I.C.*, arteria iliaca communis; *A.Il.E.*, arteria iliaca externa; *A.Is.*, arteria ischiadica; *A.L.*, arteria lumbalis; *A.L.Ma.*, arteria lienalis major; *A.L.Mi.*, arteria lienalis minor; *A.L.T.*, arteria laryngeo-trachealis; *A.M.*, arteria muscularis cervicis; *A.M.C.*, arteria mesenterica communis; *A.M.P.*, arteria mesenterica posterior; *A.O.A.*, arteria œsophagea anterior; *A.Oe.*, arteria œsophagea; *A.Pa.*, arteria parietalis; *A.P.Ma.*, arteria pancreatica major; *A.P.Mi.*, arteria pancreatica minor; *A.Pu.*, pulmonary arch; *A.Rc.*, arteria recti; *A.R.Cl.*, arteria reno-cloacalis; *A.Re.*, arteria renales; *A.S.*, arteria scapularis; *A.S.C.*, arteria subclavia; *A.Sp.*, arteria spermatica; *C.C.*, carotis communis; *C.E.*, carotis externa; *C.I.*, carotis interna; *D.A.*, ductus arteriosus (BOTALLI); *D.Ao.*, dorsal aorta; *D.C.*, ductus caroticus; *P.A.*, pulmonary arch; *S.A.*, systemic arch.

TEXT-FIG. 6.—Diagram of the Main Arteries of the Body Region in *Sphenodon*, with the alimentary canal pushed out to the right side of the animal. The dotted lines indicate approximately the position of certain of the viscera.

I have not had the opportunity of examining this vessel satisfactorily in the female, but doubtless as the ovary is disposed fairly similarly to the testis with regard to the supra-renal body the distribution of the vessel is much the same.

H. *Arteria mesenterica communis* (HOCHSTETTER) (Plate 6, fig. 1, text-fig. 6).—This vessel arises in the region of the fifteenth pair of segmental arteries, and I find that its distribution agrees more closely with the account given by HOCHSTETTER (44) than with that given by KLAATCH (50). The main trunk runs on to the upper part of the intestine and gives off a series of three or four large branches to the intestinal region. Soon after its origin it gives off a large factor (H. I) to the lower end of the intestine.

H. I. *Arteria cœcalis* (HOCHSTETTER).—After giving off a small twig to the junction of the intestine and rectum and a large one to the rectum (H. I. α) this artery runs along the end part of the intestine.

H. I. α. *Arteria recti.*—The rectal artery passes down along the dorsal wall of the rectum and anastomoses with the end of the posterior mesenteric artery.

J. *Arteriæ vasis efferentis.*—There are a series of three small vessels running to the vasa efferentia.

K. *Arteria lumbalis* (Plate 6, fig. 1, text-fig. 6).—The seventeenth or more usually the eighteenth parietal artery is much enlarged and constitutes a lumbar artery. It is distributed to the body wall and gives off two branches (K. I and K. II).

K. I. *Arteria epigastrica posterior.*—This posterior epigastric artery gives off a branch running inwards and forwards to the dorsal body wall and then itself continues on to the ventral body wall, where it turns and runs forwards and appears to anastomose with the anterior epigastric artery.

K. II. *Ramus muscularis.*—The muscular branch runs to the proximal ends of some of the muscles of the posterior surface of the thigh.

L. *Arteria iliaca communis* (GAUPP) (Plate 6, fig. 1, text-fig. 6).—This is a large artery supplying the hind limbs and it arises shortly behind the lumbar artery. It gives off an internal iliac artery (L. I) and then may be termed the external iliac artery. On reaching the limb it gives off a branch to the muscles of the proximal end of the thigh and the pelvic girdle (L. II). It then enters the leg and follows the ischiadic nerve and so may be termed the Arteria ischiadica (*i.e.*, the sciatic artery of many English text-books). Between the head of the femur and the knee it gives off several branches (L. III–VII). ZUCKERKANDL (84, p. 264) remarks that "Keine der hinteren Unterschenkelarterien kann ungeschwungen mit jenen der Säuger homologisiert werden," and the same is true also of the arteries of the thigh. Although he recognises internal and external circumflex arteries, for example, they both come off from the ischiadic and not from the femoral artery and they have not quite the

same distribution.* The account of these branches down to the knee does not quite agree with that of ZUCKERKANDL, but the difference is slight and only in matters of detail. After passing the knee the main trunk, now termed by ZUCKERKANDL the Arteria interossea, gives off a deep branch to the flexor side (L. VIII), the lateral artery of that author. He also describes a further medial artery (VII. α) coming off at this point, but I have been unable to make it out satisfactorily. Almost immediately two arteries come off in succession; the first is a circumflex (IX) and the other a posterior muscular branch (X). The interosseus then runs on to the extensor side of the limb giving off an external interosseus (XI), after which it is bridged by a ligamentum tibio-fibulare, and on to the foot where it may be termed the Arteria dorsalis pedis. This gives off a vessel, the perforans plantaris (XII), which goes through on to the flexor side of the foot and then finally the main trunk breaks up into two terminal branches, an internal (XIII) and an external (XIV).

L. I. *Arteria iliaca interna* (GAUPP).—This artery supplies several of the muscles adjacent to its origin and gives off an important branch (L. I. α).

L. I. α. *Arteria hypogastrica.*—The hypogastric artery passes ventro-mesially to the wall of the bladder.

The main trunk may here be termed the *Arteria iliaca externa* (Plate 6, fig. 1, text-fig. 6).

L. II. *Arteria glutea* (GAUPP).—The gluteal artery supplies the muscles and tissues of the gluteal region and also a part of the proximal end of the hind limb.

The main trunk here becomes the *Arteria ischiadica* (Plate 8, fig. 4).

L. III. *Arteria circumflexa femoris externa* (ZUCKERKANDL) (Plate 7, fig. 4).—The external circumflex artery is a moderate sized vessel, running to the proximal external muscles of the thigh.

L. IV. *Arteria circumflexa femoris interna* (ZUCKERKANDL) (Plate 8, fig. 4).—The internal circumflex artery is a distinct branch, supplying the adductor and other internal muscles at the top end of the thigh.

L. V. *Ramus muscularis* 1.—This artery runs down on the inner side of the ischio-tibialis muscle, and in some respects recalls the profunda artery, but, unlike it, gives off a circumflex branch (L. V. α).

L. V. α. *Arteria circumflexa genu interna.*—A small artery passes round and supplies the muscles at the lower external end of the femur.

* The arteria ischiadica is normally replaced by the arteria femoralis as the main vessel of the leg of man, but it may persist and retain its original importance in certain instructive anomalous cases, as recorded by DUBREUIL (24), KRAUSE (51), and RUGE (71).

L. VI. *Ramus muscularis* 2.—Another artery arises from the ischiadic a short distance before the knee and passes to the deeper internal muscles.

L. VII. *Arteria circumflexa genu externa* (Plate 8, fig. 4).—This circumflex artery comes off immediately above the knee and passes round its external side.

The main trunk now becomes the *Arteria interossea* (Plate 8, fig. 4).

L. VIII. *Arteria tibialis lateralis* (Plate 7, figs. 4 and 5).—This is probably the artery described by ZUCKERKANDL (*loc. cit.*) as the lateral artery, for it accompanies the nervus tibialis posticus. It runs down to the plantar surface of the foot, and takes part in the formation of the arcus plantaris, together with a branch from the external tarsal, the tibialis medialis, the perforans plantaris, and the internal tarsal. The plantar arch gives off four branches, the arteriæ interdigitales, that split over the spaces between the digits. The first of the four anastomoses with the external tarsal, and a fifth branch runs to the outside of the hallux and anastomoses with a twig from the internal tarsal. The third and fourth of these arteries are also joined by an anastomosis across the base of the third digit. This showed clearly on one specimen, but not in others, and may be an individual variation. The perforans plantaris (XII) joins the plantar arch on the radial side at the point where the fifth branch, *i.e.*, that to the outside of the hallux, is given off.

L. VIII. *α. Arteria tibialis media* (Plate 8, fig. 5).—ZUCKERKANDL describes a medial branch coming off from the interossea at this point, and running down the tibia, with a large branch of the nervus tibialis, into the depths of the foot. I have not been able to find this vessel. There is, however, a small artery which appears to be a branch of the perforans plantaris, running up the distal end of the tibia with the nervus tibialis medius. This, as will be seen, corresponds with the end of the deep-lying medial artery of ZUCKERKANDL, and so may be the same vessel.

L. IX. *Arteria circumflexa genu interna inferior.*—The inferior circumflex artery of the knee comes off just below the articulation, and supplies the adjacent internal musculature.

L. X. *Ramus muscularis* 3.—A muscular branch runs externally, and probably supplies the peroneus and other adjacent muscles.

L. XI. *Arteria interossea externa* (Ramus dorsalis, ZUCKERKANDL) (Plate 8, fig. 4).—About half way to the ankle, near the interstitium interosseum, an external interosseus artery is given off, which is related to the main interosseus much in the same way as the corresponding vessel of the fore limb. It has two terminal branches, one of which runs up and one down the limb supplying the external muscles.

The main vessel now becomes the *Arteria dorsalis pedis* (Plate 8, fig. 5).

L. XII. *Arteria perforans plantaris* (Plate 8, fig. 5) (Ramus perforans plantaris, ZUCKERKANDL).—The perforans plantaris goes through the ankle between the distal extremities of the tibia and fibula on to the plantar side. Here, as pointed out above, it gives off the tibialis media (L. VIII. α, *q.v.*), and joins the arcus plantaris near the base of the hallux.

L. XIII. *Arteria metatarsalis interna* (Plate 8, fig. 4).—The internal tarsal artery soon divides into three branches. One anastomoses with the fifth branch of the plantar arch around the external side of the base of the hallux. The other two are *Arteriæ interdigitales*, splitting over the spaces between digits one and two and two and three respectively.

L. XIV. *Arteria metatarsalis externa* (Plate 8, fig. 4).—The external tarsal artery breaks into two *Arteriæ interdigitales*, which divide over the intervals between digits three and four and four and five. The latter of these gives off a branch, which anastomoses with the first branch of the plantar arch.

M. *Arteria mesenterica posterior* (HOCHSTETTER) (text-fig. 6).—This is a small but distinct artery coming off a little posterior to the ischiadic artery, and distributed to the dorsal wall of the rectum. It gives off a ramus ascendens, which probably anastomoses with the rectal artery (H. I. α, *vide supra*).

N. *Arteria reno-cloacalis* (text-fig. 6).—The reno-cloacal artery is a small, well-marked median vessel arising shortly behind the posterior mesenteric and passing between the kidneys, to which it gives branches (N. I). Near the posterior ends of these organs the vessel divides into two branches, the cloacals (N. II).

N. I. *Arteriæ renales.*—The renal arteries consist of about three pairs of vessels, running into the substance of the kidneys.

N. II. *Arteria cloacalis.*—The cloacal artery passes outwards, and is distributed to the lateral wall of the cloaca and extreme end of the rectum. It gives off a branch, which runs to the posterior margin of the cloaca.

After giving off the reno-cloacal artery, the dorsal aorta passes into the hæmal canal of the caudal vertebræ, and so becomes the *Arteria caudalis*. It gives off small paired parietal arteries to the muscles of the tail.

The Pulmonary Arch (text-figs. 1 and 6).

The pulmonary arch, the sixth of the embryonic series and the third of those remaining in the adult, is the most posterior and the most dorsal of the three. It arises well behind the anterior ends of the atria, and runs sharply dorsalwards and backwards, giving off a branch (A) to the larynx, trachea, and œsophagus, and (B) the pulmonary artery itself. From the point of origin of the pulmonary artery, only

a short distance from the heart, the pulmonary arch continues outwards as a narrow, but nevertheless distinct, vessel, the ductus arteriosus or ductus Botalli, which runs into the systemic arch. This vestigeal vessel, whose significance has been pointed out previously (60), is present in an equally developed condition on both sides.

A. *Arteria laryngeo-trachealis* (Laryngeal artery, VAN BEMMELEN) (text-fig. 6).—This interesting vessel arises from the pulmonary arch a short distance from the heart. It was overlooked by RATHKE (66), who examined *Sphenodon*, but it was noted by VAN BEMMELEN (10), who also claims to have found it in *Platydactylus*, *Lacerta*, *Anguis*, *Pseudopus*, and *Iguana*. In company with the laryngeal nerves and the tracheal vein, it passes up the side of the trachea, to which it sends small twigs, finally breaking up in the larynx. It gives off a number of branches (I–VII).

The interest of this vessel lies in the fact that it is undoubtedly homologous with the arteria œsophagea of *Triton*, *Salamandra*, and *Spelerpes* as described by BETHGE (11). In these Urodela the respiration is carried on not only by the skin and lungs, but also by the mucosa of the buccal cavity and pharynx. Indeed, in *Speleropes*, where the lungs have been completely suppressed, it is performed entirely by the skin and the mucosa in these regions. We find as a result in this form that the pulmonary artery has disappeared, and the only branch of the pulmonary arch that remains is the very large œsophageal artery, which ramifies over the whole of the pharynx, œsophagus, and even the stomach. This relic of what is an important vessel in the Urodele Amphibia persists in certain Lacertilia as noted above, but appears to be best developed in *Sphenodon*.

A. I. *Arteria thyreoidea inferior* (VAN BEMMELEN).—This is a small artery going to the middle loop of the thyroid gland. VAN BEMMELEN (*loc. cit.*) remarks that he found this double arterial supply, partly from the pulmonary and partly from the carotid arches, going to the thyroid gland in all the Saurians he examined, but not in other Reptiles.

A. II–V. *Arteriæ œsophageæ* (Plate 7, fig. 3, text-fig. 7).—A series of small œsophageal branches are given off from the main vessel as it ascends the neck.

A. VI. *Arteria anastomotica* (text-fig. 7).—This is a small artery which anastomoses with its fellow ventrally to the posterior end of the larynx.

A. VII. *Arteria laryngea inferior* (text-fig. 7).—The inferior laryngeal artery breaks up into a number of twigs supplying the larynx and the genio-glossus muscle.

B. *Arteria pulmonalis.*—The pulmonary artery arises from the arch a short way beyond the laryngeo-tracheal artery and runs down the dorso-lateral border of the lung to which it is attached.

The Venous System.

Considering the work that has been done on the vascular system of the Reptiles in general it is astonishing that there is nothing in the nature of a complete description of the venous system of *Sphenodon*, or of any Lacertilian. Even Corti, who has given such a detailed account of "*Psammosaurus griseus*," dismisses the veins in a couple of pages, remarking that he has neither successfully injected them nor dissected them out. Certain points have been dealt with in a fairly full manner, however.

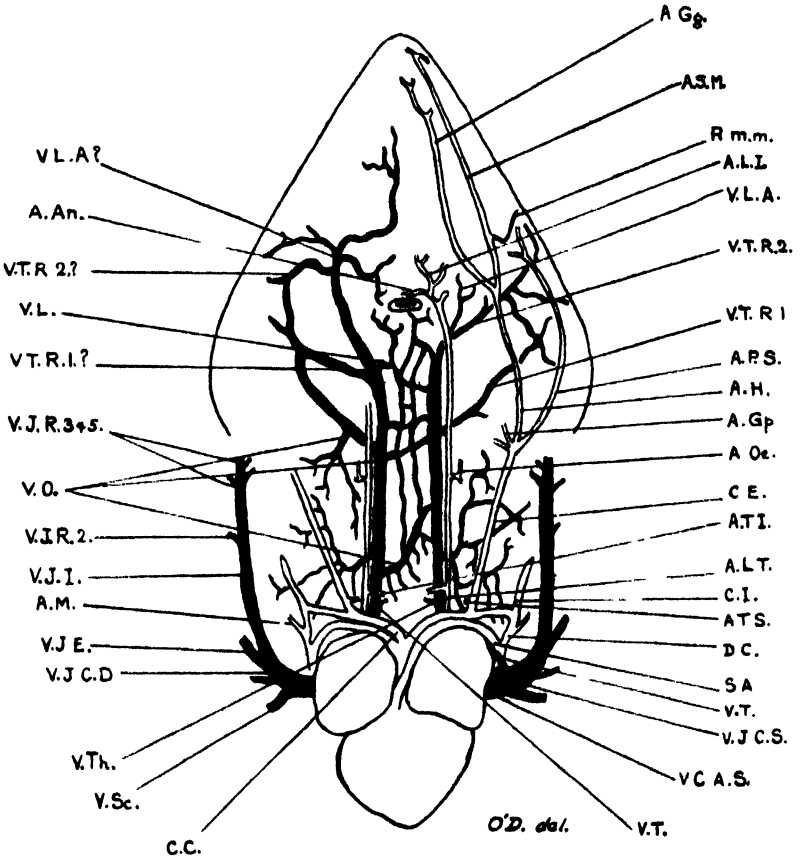
Rathke (65) again provided the starting point of our modern knowledge by his account of the development of *Tropidonotus natrix*. This was added to by the work of Hofmann (47) on the development of the venous system in *Lacerta agilis*. Hochstetter (46) has dealt with the development of the veins in both *Lacerta* and *Tropidonotus*, but confined his attention mainly to the veins posterior to the heart, confirming and extending the observations of Hofmann. The development of the veins in the head and neck, again in *Lacerta* and *Tropidonotus*, have also been well described by Grosser and Brezina (40). Further, we have the interesting work on the cephalic vessels in *Lacerta agilis*, *Tropidonotus natrix*, and *Emys Europæa* by Bruner (18), in which he describes not only the vessels of the adult very fully, but also a muscular mechanism connected with them, whereby the venous blood pressure in the head can be raised. This is apparently called into action during the process of sloughing the skin. In addition to these more or less particular studies, a great number of comparative points have been cleared up in a valuable series of papers by Beddard (2–5, 7 and 8). Lastly, concerning *Sphenodon* itself, we have the work of Dendy (22) on the intra-cranial vessels, including the veins, so that as in the case of the arteries it is only necessary to indicate the relationship of the extra-cranial trunks to them. There is also a description of the sinus venosus in a paper by Röse (70).

As has already been indicated in dealing with the heart, the three main veins of the body, the two anterior and the single posterior venæ cavæ open into a distinct sinus venosus. The two procaval veins are similar in origin, and as the only differences between them are in the relative sizes of their constituent veins, it is only necessary to describe one of them in detail. In dealing with the venous system, the plan is often adopted of commencing with the smallest vessels and working gradually through the larger trunks to the heart. While this has certain advantages and indicates the direction of the blood flow, it is not the general method pursued in actual dissection. The vessels are usually found by following up the larger trunks away from the heart towards the periphery, and that is the plan adopted in the following description.

Vena cava anterior sinistra (Plate 7, fig. 3, text-fig. 7).—The left precaval vein is a short stout trunk opening into the dorsal side of the sinus venosus, and formed a

short distance from the heart by the union of three veins, the tracheal (A), the common jugular (B), and the sub-clavian (C).

A. *Vena trachealis* (GROSSER and BREZINA, BRUNER) (text-fig. 7).—The left tracheal vein runs from its point of entry into the left precaval vein, dorsal to the systemic and carotid arches, up the left side of the trachea. It receives tributaries (A. I–X) on its way to the larynx, some of which anastomose with their fellows of the other side and produce a venous network on the ventral side of the œsophagus. In the laryngeal region it receives further branches (XI–XII) from the muscles of the floor of the mouth. The constitution of the right tracheal vein is on the whole similar to that



TEXT-FIG. 7.—Diagram to show the distribution of the Venæ Tracheales and adjacent Arteries. The trachea has been cut off close to the larynx and removed.

A.An., arteria anastomotica; *A.Gg.*, arteria genioglossa; *A.Gp.*, arteria glossopharyngeus; *A.H.*, arteria hyomandibularis; *A.L.I.*, arteria laryngea inferior; *A.L.T.*, arteria laryngeo-trachialis; *A.M.*, arteria muscularis cervicis; *A.Oe.*, arteria œsophagea; *A.P.S.*, arteria pterygoideus superficialis; *A.S.M.*, arteria sub-mandibularis; *A.T.I.*, arteria thyreoidea inferior; *A.T.S.*, arteria thyreoidea superior; *C.C.*, carotis communis; *C.E.*, carotis externa; *C.I.*, carotis interna; *D.C.*, ductus caroticus; *R.m.m.*, ramus musculo-mandibularis; *S.A.*, systemic arch; *V.C.A.S.*, vena cava anterior sinistra; *V.J.C.D.*, vena jugularis communis dextra; *V.J.C.S.*, vena jugularis communis sinistra; *V.J.E.*, vena jugularis externa; *V.J.I.*, vena jugularis interna; *V.J.R.I.*, rami musculares of v. jugularis interna; *V.L.*, vena laryngea; *V.L.A.*, vena laryngea anterior; *V.O.*, venæ œsophageæ; *V.Sc.*, vena subclavia; *V.T.*, vena trachealis; *V.Th.*, vena thyreoidea; *V.T.R.* 1 and 2, rami musculares of v. trachealis.

of the left, but the former is considerably larger and has more branches. In the embryo of *Lacerta*, according to both BRUNER (18) and GROSSER and BREZINA (40), a tracheal vein is present on both sides. During the course of development, however, anastomoses arise between them, no doubt corresponding to those in the adult *Sphenodon*, which allow of the complete disappearance of the posterior portion of the left vein in later stages. Thus it follows that in the adult only the right tracheal vein persists, as pointed out by PARKER (64), who, however, calls it quite wrongly (p. 161) the external jugular vein, and by VOGT and JUNG (78), who term it "die unpaare Kopfvene" (p. 714).

The condition in *Sphenodon* is noteworthy, since it is more primitive than that in *Lacerta*, and, moreover, represents a stage passed through by the latter animal in the course of its development. Further, the left vein in *Sphenodon* is much smaller than the right, as if foreshadowing its partial disappearance in other forms.

The vena trachealis in *Sphenodon* and *Lacerta* is an interesting vein, for, as will be seen from the above account, its distribution is very similar to that of the external jugular of the Amphibia, but at the same time it is obviously not the same vessel. In the frog, as is generally known, and in *Salamandra*, *Triton*, and *Spelerpes*, according to BETHGE (11), the external jugular is a fairly superficial vein running *ventrad* of the arterial arches and receiving two main tributaries, the vena lingualis and vena mandibularis. The tracheal vein in *Sphenodon* passes *dorsad* of the arterial arches and does not receive two such definite vessels, although it drains very nearly the same territory. There is present in the three Urodeles a vessel from which it seems easily possible to derive the vena trachealis of the Saurians, and that is the vena pharyngea (BETHGE). This vessel arises posterior to the arterial arches and its course lies dorsal to them along the wall of the pharynx. It will easily be seen then that anastomoses between it and the lingualis would enable it to take over the latter and through it the mandibularis, thus reducing the vena jugularis externa in *Sphenodon* (*q.v.*) to the unimportant vessel that it is.

A. I. *Vena thyreoidea* (text-fig. 7).—The thyroid gland is drained by a small vein entering the tracheal vein near its proximal end.

A. II–V. *Venæ œsophageæ* (text-fig. 7).—A number of veins come from the walls of the œsophagus. Some come inwards from the lateral wall and others outwards from the ventral wall. The latter take part in the formation of the venous network already described.

A. VI. *Ramus muscularis* 1.—This is a vein coming from the inner surface of the internal pterygoid muscle.

A. VII–X. *Venæ laryngeæ*.—The laryngeal veins are a series of small branches coming from the posterior muscles of the larynx. They anastomose with

similar vessels from the right, and also have twigs from the anterior end of the œsophagus running into them.

A. XI. *Ramus muscularis* 2.—This is a fair-sized vein, running outwards from the side of the larynx and receiving three tributaries :—

XI. α. A branch that anastomoses with A. VI.

XI. β. A branch coming backwards from the lateral surface of the internal pterygoid muscle.

XI. γ. A branch coming forward from the same surface of the internal pterygoid muscle.

A. XII. *Vena laryngea anterior* (text-fig. 7).—The anterior laryngeal vein bends round the lateral wall of the larynx and receives three main branches :—

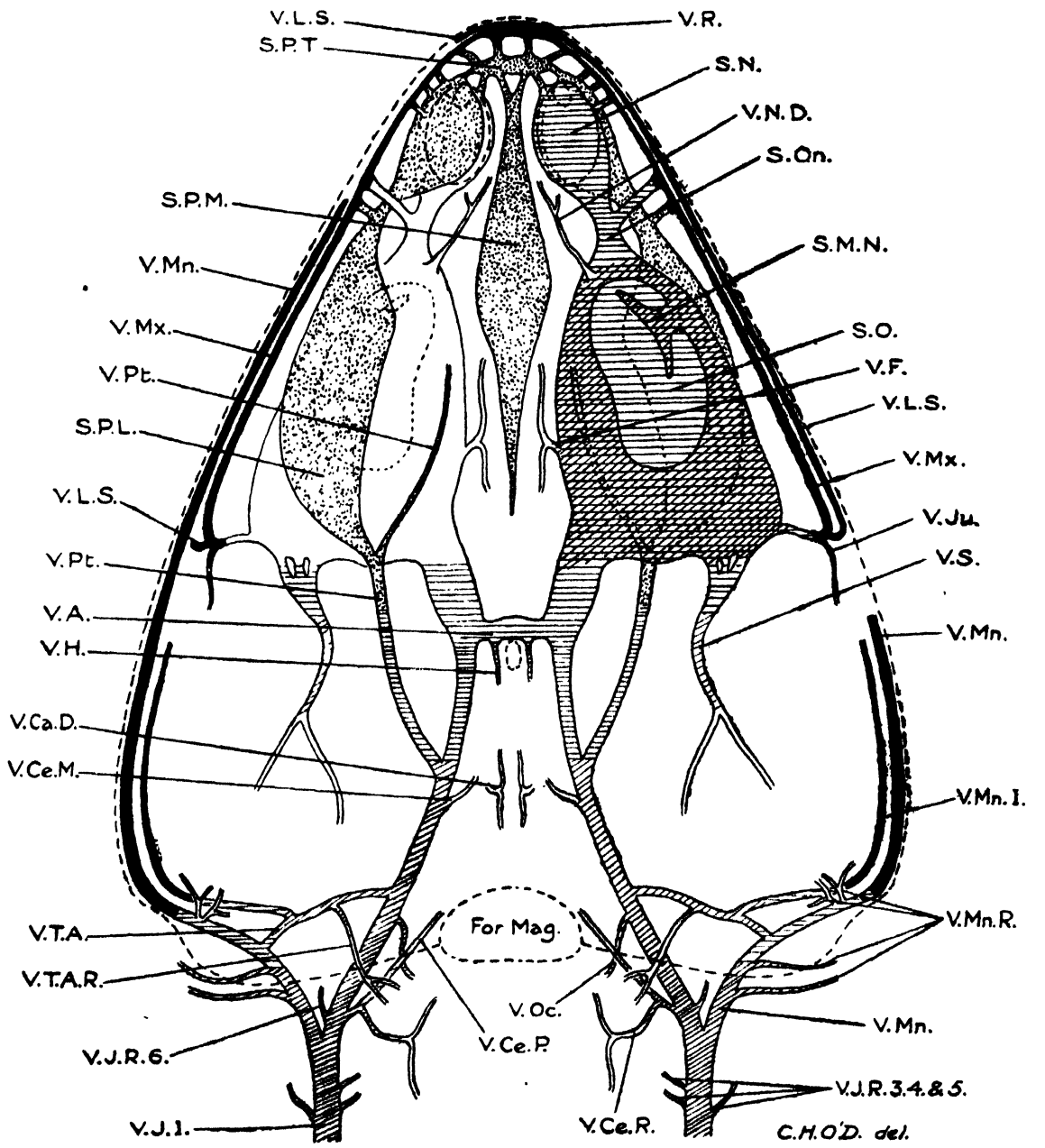
A. XII. α. A branch from the lateral and anterior walls of the larynx.

A. XII. β. A branch from the cerato-mandibular muscle.

A. XII. γ. A branch from the genio-glossus muscle.

B. *Vena jugularis communis sinistra* (V. jugularis, Corti) (Plate 7, fig. 3, text-fig. 7).—The left common jugular vein is the second of the three trunks that unite to form the left precaval. From its origin it passes slightly laterally and then up the neck, receiving after a short distance a muscularis branch (B. I) and, a little more anteriorly, the external jugular (B. II). From this point it is continued forward as the *Vena jugularis interna* (V. jugularis, Grosser and Brezina ; V. jugularis interna, Bruner), receiving a number of branches (B. III–VI) up to the hinder end of the neck, where it is crossed dorsally by the tenth cranial nerve and the internal carotid artery. Just at this point it receives the large mandibular vein (B. VII). A very short distance in front of this it receives a branch (B. VIII) on its outer side, and on the inner side, at the same level or just anterior to it, the posterior cerebral vein (B. IX). This is an important landmark, as the combined vessel formed by the main trunk up to this point and then continued on as the posterior cerebral vein is homologous with the internal jugular vein of the mammals, including man.

The constitution of the anterior end of the main trunk can only be properly understood in the light of its development. A marked difference between the venous supply in the head of the human embryo and of the adult was first pointed out by Luschka (53), who, however, was not in possession of sufficient data to give a satisfactory explanation thereof. This line of enquiry was carried further by Salzer (72), who worked out the partial replacement of the old "anterior cardinal vein" (*sic*, really vena capitis medialis) in the head region by a more laterally situated trunk, which he termed the vena capitis lateralis. A further paper by



TEXT-FIG. 8.—Diagram of the Venous System of the Head. Vessels in jaws indicated in black. Palatine vessels dotted shading. Vessels at level of ventral side of cranium horizontal shading. Vessels at higher level oblique shading, and most dorsal vessels in outline only. On right side the mandibular vein is cut off short behind level of maxillary vein. The orbital and orbito-nasal veins are fully displayed. On left side the superior labial vein is cut off short at its origin, and the palatine sinuses fully shaded, while the orbital and orbito-nasal sinuses are indicated in outline or dotted outline only.

For.Mag., foramen magnum; *S.M.N.*, sinus membranæ nictitantis; *S.N.*, sinus nasalis; *S.O.*, sinus orbitalis; *S.On.*, sinus orbito-nasalis; *S.P.L.*, sinus palatinus lateralis; *S.P.M.*, sinus palatinus medius; *S.P.T.*, sinus palatinus transversus; *V.A.*, vena anastomotica; *V.Ca.D.*, vena capitis dorsalis; *V.Ce.M.*, vena cerebralis media; *V.Ce.P.*, vena cerebralis posterior; *V.Ce.R.*, ramus muscularis of vena cerebralis posterior; *V.F.*, vena frontalis; *V.H.*, vena hypophyseos lateralis; *V.J.I.*, vena jugularis interna; *V.J.R.* 3, 4 and 5, rami musculares of vena jugularis interna; *V.J.R.* 6, ramus muscularis of vena jugularis interna; *V.Ju.*, vena jugalis; *V.L.S.*, vena labialis superior; *V.Mn.*, vena mandibularis; *V.Mn.I.*, vena mandibularis interna; *V.Mn.R.*, rami musculares of vena mandibularis; *V.Mx.*, vena maxillaris; *V.N.D.*, vena nasalis dorsalis; *V.Oc.*, vena occipitalis; *V.Pt.*, vena pterygoidea; *V.R.*, vena rostralis; *V.S.*, vena supratemporalis; *V.T.A.*, vena tympanica anterior; *V.T.A.R.*, ramus muscularis of vena tympanica anterior.

GROSSER and BREZINA (40) gave an excellent description of the development of the vascular system of *Lacerta* and *Tropidonotus*, and lastly GROSSER (39) reviewed these changes in the vertebrate series. As far as these results apply to Reptilia and particularly Lacertilia, they may be briefly epitomised as follows :—In the fairly early embryo there are present two longitudinal veins in the head and neck. The first is the anterior cardinal vein, probably to be regarded as intimately related to the somites, and starting at their anterior end behind the auditory vesicle. It runs backwards into the Ductus Cuvieri. The second, the vena capitis medialis (GROSSER and BREZINA), lies on the base of the brain ventral to and slightly mesial to the primordia of the cranial nerves, and receives the orbital sinus. These two are connected by means of a more or less transverse vessel, the vena cerebralis posterior (GROSSER and BREZINA), shortly behind the primordium of the tenth cranial nerve. As development proceeds a series of vascular rings are laid down around the primordia of cranial nerves five to ten. The linking up of the outer portions of these rings constitutes another longitudinal venus trunk, the vena capitis lateralis (SALZER), which lies dorsal and slightly lateral to the nerve roots. The vena cerebralis posterior grows upwards and joins with its fellow to help to form the vena longitudinis cerebri, which also acquires two further connections, with the vena capitis medialis and later with the vena capitis lateralis. The hinder of these two connections, the vena cerebralis media (GROSSER and BREZINA), runs round behind the cerebellum and into the vena capitis medialis close by the ganglion of the trigeminus. The front vessel, the vena cerebralis anterior (GROSSER and BREZINA), begins at the epiphysis cerebri and passes behind the cerebral hemisphere into the vena capitis medialis just behind the orbit.

With the subsequent disappearance of the inner longitudinal vessel, the vena capitis medialis, and of the transverse portions of the venous rings, the definitive head vessel comes to consist of three parts. 1. The posterior part, derived from the old anterior cardinal vein, extends up to the vena cerebralis posterior. 2. The median portion from this point on to just in front of the ganglion of the trigeminus is derived from the vena capitis lateralis. 3. The remaining anterior part is the remnant of the vena capitis medialis and the orbital sinus.

The vessel so far described in the adult *Sphenodon* is the posterior part, *i.e.*, the persistent anterior cardinal. The *vena capitis lateralis* portion passes upwards and inwards to the posterior end of the skull, running under the paroccipital bone, dorsal to the columellar auris and dorso-mesial of the bifurcation of the stapedial artery, to form the temporal and mandibular arteries. Just behind the columella auris the occipital vein (B. X) enters the vena capitis lateralis, and in front of the columella it receives the anterior tympanic (B. VII. γ), which puts it in communication with the mandibular vein. It continues forwards under the paroccipital bone along the ventro-lateral wall of the auditory capsule, on which

its position is faintly marked by a shallow inconspicuous groove, through the foramen between the quadrate wing of the pterygoid bone and the otic capsule (*i.e.*, the foramen for the hyomaxillary branch of the seventh cranial nerve, and termed by WATSON (79) in *Diademodon* the pterygo-paroccipital fossa). Passing dorsal to the roots of the facialis nerve it receives on its dorsal side the median cerebral vein (B. XI) just behind the splitting of the trigeminus, and this marks the end of the vena capitis lateralis portion.

The main trunk, now in its anterior or *vena capitis medialis* portion, runs on ventral of the roots of the trigeminus forwards near the mid-ventral line towards the orbital sinus. A short distance beyond the nerve the pterygoid vein (B. XII) enters the vena capitis medialis, and at the level of the front end of the hypophysis cerebri it receives an anastomosing vein (B. XIII) which puts it into communication with its fellow of the opposite side. Anterior to this the character of the vessel changes, and, from being a vessel with a definite wall, it becomes sinusoidal in nature. The actual vessel can be traced forwards for some distance on the dorso-mesial aspect of the musculus protrusor oculi, as figured by BRUNER (18) in *Lacerta*, but the orbital sinus extends postero-lateral of this and somewhat obscures the vessel. The sinuses ultimately merge into one large *Orbital sinus*, which, although a continuation of the main trunk, can be more conveniently dealt with separately. For the whole of this main venous trunk BRUNER (*loc. cit.*) employs the term vena jugularis interna, but, as pointed out above, only part of it is homologous with the similarly named trunk in the mammals, and, in front of that, we have to deal with at least two portions that are distinct developmentally.

B. I. *Ramus muscularis* 1.—This is a small vessel joining the common jugular close to the heart and coming from the adjacent dorso-lateral muscles. It is not present on the right side of one specimen with the venous system injected, and so may not be constant.

B. II. *Vena jugularis externa* (BRUNER) (Plate 6, fig. 1; Plate 7, fig. 3, text-fig. 7).—There is a well marked vessel joining the internal jugular low down in the neck to form the common jugular vein. This vessel, from its general disposition, is apparently the external jugular vein, but this cannot be regarded as definitely established without reference to its development, for, as has been pointed out previously, it has apparently yielded its lingual and mandibular branches to the tracheal vein (*q.v.*). I have not been able to ascertain whether it is connected with the external mandibular, as BRUNER (18) states the corresponding vessel is in *Lacerta*. This is not improbable, however, since it runs right up the neck ventro-laterad of the cervical nerves as far as the posterior region of the head, draining the superficial muscles and subcutaneous tissue.

The diminution in the functional importance of the external jugular vein in *Sphenedon* and *Lacerta* is interesting, since that vessel is of fair importance in the Amphibia, and is still more important in the Mammalia, where, if it is the same vessel, as seems probable, the external jugular drains the whole of the head and face, leaving practically only the brain to the internal jugular. In spite of the works of SALZER (72), and MALL (55), and SMITH (74), the question of the development of the external jugular vein in the Mammal does not appear to have been satisfactorily worked out. It is not merely that the external jugular in this order retains the factors that it has in the Amphibia, but it also adds to them in some way or other all the territory drained by the capitis lateralis and capitis medialis portions of the main head vein in such a form as *Sphenodon*.

As will be seen from the above accounts, the names Internal and External jugular vein have a very different significance in the different groups of Vertebrates, and the terms should not be employed unless carefully defined.

From this point the main trunk is the anterior cardinal portion of the vena jugularis interna.

B. III. *Ramus muscularis* 2 (Plate 7, fig. 3, text-fig. 7).—This is a vein coming from the cucullaris muscle. It receives a well marked factor, which comes in from the surrounding subcutaneous tissue and penetrates the muscle.

B. IV. *Ramus muscularis* 3.—This is a second twig, draining the anterior part of the cucullaris muscle.

B. V. *Ramus muscularis* 4.—A small vessel returns blood from the longissimus dorsi muscle, and probably also from the deeper layers of the cucullaris.

B. VI. *Ramus muscularis* 5 (Plate 7, fig. 3, text-fig. 7).—Another fairly small vessel comes from the antero-dorsal region of the cucullaris muscle.

B. VII. *Vena mandibularis* (V. maxillaris inferior, GROSSER and BREZINA; V. mandibularis, BRUNER) (text-fig. 8).—The mandibular vein is a well marked tributary, which joins the internal jugular at the point where the latter is crossed by the vagus. It is undoubtedly the same vessel as that described and figured by GROSSER and BREZINA (40), who, however, term it the inferior maxillary, a somewhat misleading name, since it has nothing to do with the maxilla. It passes outwards internal to the anterior cornu of the hyoid and posterior to the mandible, receiving two branches (B. VII. α and β). Just internal to the quadrato-jugal bone, an important tributary, the anterior tympanic vein (B. VII. γ) joins it, and, shortly after this, two twigs (B. VII. δ and ϵ) from the masetter muscle. The main trunk then takes its course along the outer surface of the mandible, finally entering the substance of the bone through a small foramen situated at the end of a short shallow groove. Just before so doing it receives a small external branch.

At this point it seems advisable to call attention to the interesting work of BRUNER (18), who has described a neuro-muscular apparatus connected with the bases of the vena mandibularis and the vena cerebralis posterior and the neighbouring part of the main trunk of the vena jugularis interna in various Lacertilia, Testudinata and Ophidia. It takes the form of a well-developed external muscular sheath, "the musculus constrictor venæ jugularis internæ," enervated by a special nerve supply, whereby the three vessels can be considerably constricted, and the venous pressure in the head greatly increased. The apparatus is useful, apparently, in helping in the operation of shedding the skin. The muscles are fairly easily seen in *Lacerta* and *Tropidonotus*, but are not quite so obvious in *Sphenodon;* they can be made very plain, however, in the following way. If the anterior region of the internal jugular, with a good piece of the ends of both mandibular and posterior cerebral veins, be dissected out, and any coagulated blood in them washed out and the preparation placed in a glass dish of alcohol containing a little glycerine, the muscles can readily be made out. When viewed by transmitted light, the ordinary parts of the vein wall appear moderately transparent and thin-walled, but, in the region occupied by the muscle, the walls of the vessels are very much thicker and more opaque. Transverse sections through the various regions also bring out quite clearly the fact that the bases of both mandibular and posterior cerebral vein, and of the internal jugular in the vicinity, are enveloped by a conspicuous coat of striate muscle, just as BRUNER described in other Saurians. The condition of the specimens did not allow of detailed examination of its nerve supply, but, as it lies quite close to the ganglion glossopharyngei, as in *Lacerta*, the nerves to the muscle, nervi tumefactores capitis, are doubtless fairly similar. For the same reason, the connection of the muscle with the skeleton, if present, could not be satisfactorily ascertained.

I also find myself in agreement with BRUNER with regard to the late appearance of these muscles during the course of development, for they are but feebly shown in the oldest stage I have examined (Stage S of DENDY).

B. VII. α and β. *Rami musculares* a and b.—These are two small muscular branches entering the mandibular vein in the region of the angle of the jaw from which they come.

B. VII. γ. *Vena tympanica anterior* (BRUNER) (Plate 7, fig. 2, text-fig. 8).—The anterior tympanic vein is an important tributary joining the mandibular a little further along its course. It passes mesially through the ear cavity anterior to the columella but behind the quadrate to enter the vena capitis lateralis just anterior to the point where it is crossed by the stapedial artery. Thus it forms an anastomosis between the mandibular and lateral head veins. In the region of the mandible it receives the internal mandibular (VII. γ. i), and dorso-mesiad of this a ventrally running branch (VII. γ. ii).

VII. γ. i. *Vena mandibularis interna* (BRUNER) (Plate 7, fig. 2, text-fig. 8).—The internal mandibular vein after leaving the anterior tympanic runs outwards below the quadrate bone and forward along the inner surface of the mandible, which it drains.

VII. γ. ii. *Ramus muscularis*.—This is a fair sized vessel bringing back blood from the anterior end of the longissimus muscle and the tissues in the region of the post temporal fossa.

B. VII. δ and ϵ. *Rami musculares* c and d.—These are two veins which almost immediately re-divide and drain the main mass of the temporo-masetter muscle.

B. VIII. *Ramus muscularis* 6.—This is a short branch from the parieto-mandibularis muscle.

B. IX. *Vena cerebralis posterior* (GROSSER and BREZINA; V. cephalica posterior, VERSLUYS and DENDY) (Plate 2, fig. 2, text-fig. 8).—The posterior cerebral is a small but important vein which passes upwards and inwards from the internal jugular under the cucullaris muscle from which it receives a branch (B. IX. α). It takes up a position near the root of the tenth cranial nerve, and with it enters the skull through the jugular foramen. The distribution of this vein and its relations to other vessels within the cranium, where it receives a large posterior factor, the vena spinalis, has been dealt with fully by DENDY (22).

This last author proposes to call this vessel the *vena cephalica posterior*, following VERSLUYS (77), on the grounds that cerebralis is undesirable, as it has "nothing to do with the cerebrum proper" (*loc. cit.*, p. 413). I think on the whole it is better to retain the original terminology of GROSSER and BREZINA for several reasons. As a matter of fact, the adjective cerebral is usually employed in comparative anatomy to indicate a structure related to the brain as a whole, *e.g.*, cerebral carotid, while the term cephalic is used for a vessel related to the head. Thus, to extend the same nomenclature to the second of the transverse brain vessels, the *vena cerebralis media* of GROSSER and BREZINA, would lead to confusion, for median cephalic vein is a much closer translation of the *vena capitis medialis* of these authors, which is a totally distinct vessel. In the second place, VERSLUYS used the term *vena cephalica posterior* in error, probably misreading the lettering in the figures of the original authors, for he says (*loc. cit.*, p. 349): "Die andere kommt durch das Foramen magnum, dann zwischen Schädel und Atlasbogen hindurch aus der Schädelhöhle; GROSSER u. BREZINA haben sie 'Vena cephalica posterior' genannt." These investigators, however, nowhere use the term cephalica, but call the vessel in question the v. cerebralis posterior. Lastly, several subsequent writers, including GROSSER himself, in a comparative account of the head veins of Vertebrates (39), have employed the term cerebralis, which is now fairly widely accepted.

A rather striking difference is to be noted between the situation of the vena cerebralis posterior in *Sphenodon* and in *Lacerta* and *Tropidonotus*. In *Sphenodon*, as has been pointed out, the vein in question leaves the cranial cavity through the jugular foramen, and is in consequence homologous with the so-called internal jugular of mammals and birds. Both VERSLUYS (77) and GISI (34) state that they have been unable to find the posterior cerebral vein in *Sphenodon*, although the former points out that the canal leading from the foramen jugular is wider than would be necessary for the nerve alone. SCHAUINSLAND (73), however, has described the presence of a vein passing through the skull by the jugular foramen in company with the tenth and eleventh cranial nerves. As pointed out by DENDY (22), whose statement I am able to confirm, there is no doubt that the posterior cerebral vein leaves the skull through the foramen jugulare.

VERSLUYS (77), GROSSER and BREZINA (40), and BRUNER (18) are all in agreement that the posterior cerebral vein in *Lacerta* and *Tropidonotus* leaves the cranial cavity by the foramen magnum and not through the foramen jugulare. CLASON (20A) describes in *Lacerta* a fine vein passing through the jugular foramen, but this appears to be either an error in observation or the lizard in question was slightly abnormal, for, as will be seen later, this is also a possible explanation. HASSE (43) in *Tropidonotus* describes a vessel coming out through the foramen jugulare, but this has not been found by subsequent workers, *e.g.*, GROSSER and BREZINA (*loc. cit.*), BRUNER (*loc. cit.*), and O'DONOGHUE (58). It is stated that in all the Lacertilia dissected by VERSLUYS (*loc. cit.*), the vena cerebralis posterior came out by the foramen magnum with the single exception of *Amphisbæna fuliginosa*, in which there was also present a vein issuing from the jugular foramen. The two veins in this species enter the vena jugularis interna. This condition is an interesting one, for it is also realised in *Emys Europæa* (BRUNER), and as GROSSER and BREZINA point out is to be found in *Lacerta* embryo with a head length of 4·1 mm. A similar arrangement is probably to be found in the Crocodile, since RATHKE (68) mentions a vein leaving the cranial cavity in company with the tenth nerve, and another through the foramen magnum. His description is not at all easy to follow, however, and I agree with DENDY that the point must await further investigation. This loop on the posterior cerebral vein shows how it is possible for the two different conditions met with in *Sphenodon* and the Lacertilia to be arrived at, for it simply becomes a question of which part of the loop persists in the adult.

In Elasmobranchs the posterior cerebral vein leaves the skull with the tenth cranial nerve, as was pointed out in the embryo by GROSSER (39) and in the adult by O'DONOGHUE (59). The same happens in both Urodele and Anurous Amphibia according to REX (69), although there is some variation in the relative sizes of the venæ cerebrales posterior and media in this class. Furthermore, we find the vein leaving the skull with the tenth nerve in the Chelonia, according to HASSE (43), and as noted above also in Mammalia and Aves. There can be little doubt, then, that the

condition in *Sphenodon* in this respect is more primitive than in the Lacertilia and Ophidia, and that it approximates more closely to the Urodele Amphibia.

Thus, although a vein of moderate calibre, the vena cerebralis posterior is of considerable morphological importance, since it is one of the most constant veins in the head of Vertebrata and serves as a valuable landmark.

B. IX. α. *Ramus muscularis.*—On its way beneath the cucullaris muscle, the posterior cerebral vein receives a branch from that muscle. It is formed by the union of an interior and posterior factors.

The main vessel now enters on its *Vena capitis lateralis* portion (Plate 7, fig. 2).

B. X. *Vena occipitalis* (Pate 7, fig. 2, text-fig. 8).—The occipital vein runs inwards and downwards from the lateral head vein, which it joins just behind the columella. It returns the blood from the muscles and tissues in the occipital region.

A short distance in front of this the main trunk receives the vena tympanica anterior, B. VII. γ, linking it up with the mandibular vein.

B. XI. *Vena cerebralis media* (GROSSER and BREZINA; Sinus transversus, DENDY) (Plate 7, fig. 2, text-fig. 8).—The intercranial course of this vessel has been dealt with by DENDY, who also describes its passage through the membranous cranial wall and downwards behind the epipterygoid bone into the end of the vena capitis lateralis just by the ganglionated root of the trigeminus. Intra-cranially it enlarges to form a big triangular sinus, and receives a branch, the vena capitis dorsalis (XI. α). BRUNER (18) describes in the adult *Lacerta* a secondary extra-cranial connection between the median cerebral vein and the orbital sinus. I have not been able to discover any such vein in *Sphenodon.*

Although part of the median cerebral vein is extra-cranial in *Sphenodon*, it lies in close apposition to the membranous cranial wall down to the root of the trigeminus. There seems to be little doubt that it is completely homologous with the vein leaving the cranial cavity with the fifth nerve in such a form as *Ornithorhynchus*, in which the disappearance of the lateral membrane and the formation of a more external bony cranial wall has caused it to become completely intra-cranial. It appears to be a fairly widespread vein, as it occurs generally in the Amphibia, where it sometimes forms the main vessel leaving the cranium, and also in early Reptilia. In *Diademodon*, for example, it probably left the skull through the foramen described by WATSON (79, p. 303) for the posterior two roots of the fifth nerve lying at the front end of the groove leading forward from the pterygo-paroccipital foramen.

B. XI. α. *Vena capitis dorsalis* (BRUNER; DENDY) (text-fig. 8).—The dorsal head vein drains the muscles in the spino-occipital region, and just before

piercing the skull to enter the median cerebral vein it receives an anterior factor from above the parietal bone, the *vena parietalis* of BRUNER. Apparently it passes through the skull by a small foramen in the parietal bone or between it and the pro-otic or supra-occipital regions. The exact position of this cannot be made out with certainty in an old skull. It enters the sinus transversus near its origin from the sinus (vena) longitudinalis. Mr. WATSON informs me that the entrance of a vena capitis dorsalis through a foramen or notch in the side of the hinder end of the brain case, either in the pro-otic or supra-occipital, appears to be a constant feature in early Amphibia and Reptilia, and the foramen is figured by him in Diademodon (79, p. 301). This is also noted by ANDREWS (1, p. 87) in Stenosaurus.

The main trunk now enters on its *Vena capitis medialis* portion.

B. XII. *Vena pterygoidea* (BRUNER) (Plate 7, fig. 2, text-fig. 8).—The pterygoid vein joins the lateral cephalic just in front of the trigeminus and the epipterygoid bone. It runs forwards and outwards in company with the palatine branch of the facial nerve and the palatine artery to the region of the sub-orbital foramen, through which it receives a large branch (XII. α). The main vessel continues on, passing towards the middle line again, and drains the dorsal surface of the palatine bone. The details of the finer connections at the anterior end cannot be made out satisfactorily by dissection, but are visible in a series of transverse and longitudinal sections of late embryos that Prof. DENDY kindly placed at my disposal.

B. XII. α. *Sinus palatinus lateralis* (BRUNER) (text-fig. 8).—The lateral palatine sinus enters the pterygoid vein near the sub-orbital foramen, through which it passes on to the ventral side of the palatine bone. At first a single trunk, it soon forms a sinusoidal network in the sub-mucosa of the palate, passing forward mesiad of the inner tooth row. Beneath the front end of the orbit it again runs together to form a single trunk, only to swell out shortly afterwards to form a large sinus beneath the nasal capsule. In this part of its course it anastomoses with the maxillary vein. Finally, right at the front end of the pre-maxillary region we find it takes its origin in a transverse sinus (XII. α (i)).

XII. α (i). *Sinus palatinus transversus* (BRUNER).—The transverse palatine sinus is a small vessel joining the two lateral palatine sinuses across the middle line, but it is important because of its connections. Laterally it runs into the maxillary vein, and through it is put into communication with a still more anterior transverse trunk, the rostral vein (B. XX). In the middle line the transverse palatine sinus receives the two openings of the median palatine sinus (α (i) a).

α (i) a.* *Sinus palatinus medius* (BRUNER).—The median palatine sinus opens by two veins, which soon unite to form a single vessel. This passes back in the middle line and in the mid-orbital region forms a network of veins. Small vessels connect it with the lateral palatine sinus here and there.

B. XIII. *Vena anastomotica* (text-fig. 8).—This anastomosing vein joins the two median cephalic veins as they run along the membranous wall of the cranium just in front of the pre-sphenoid and in front of the hypophysis cerebri. It is a short vessel receiving on each side a branch—the lateral hypophyseal vein (B. XIII. α).

B. XIII. α. *Vena hypophyseos lateralis* (text-fig. 8).—This vein is here called lateral to distinguish it from the vena hypophyseos of BRUNER, which according to that author is connected with the median cerebral vein. It passes backwards inside the membranous wall of the hypophyseal cavity and apparently joins its fellow just behind the pituitary body. DENDY (22) was not able to find any vessel corresponding to the hypophyseal of BRUNER in the adult, nor does it appear to be present in the embryo.

Sinus orbitalis (BRUNER) (Plate 7, fig. 2, text-fig. 8).—Almost immediately in front of the anastomosis, the median cephalic vein becomes enlarged and sinus-like and so constitutes the orbital sinus. The foundation of our knowledge of this region in the Reptilia was laid by WEBER (80), although he took no notice of the veins leading into it. The exact limits of the sinus and point of entry of its tributaries are difficult to make out by dissection, but I have been much aided by the series of sections already mentioned. It is a very large vessel, extending from just in front of the anterior end of the hypophysis, where its hinder limits are marked by the fascia separating it from the temporal fossa, forward to the orbito-nasal septum. Through the fissura orbito-nasalis of GAUPP (32) it communicates with the nasal sinus (B. XIX. α). Its floor is formed by the smooth orbital muscle similar to that described by LEYDIG (52) in Lacertilia, and its internal boundary is the septum interorbitale. The sinus therefore occupies practically the whole of the orbit not taken up by the eyeball and its muscles, but it is incomplete dorsally save at the posterior end. Antero-mesially, although it passes up the septum, it only runs for a very short way along the roof of the orbit, and the same occurs antero-laterally, so that the sinus is much smaller at the front than at the back end of the orbit. Although it does not lie above the eyeball at the anterior end, it is in communication there with a subsidiary sinus in the nictitating membrane (B. XVIII). The main sinus by means of a series of tributaries (B. XV–XX) drains almost the whole of the anterior region of the head with the exception of the palate, and it also receives a posterior trunk from the supra-temporal region (B. XIV).

B. XIV. *Vena supra-temporalis* (BRUNER) (Plate 7, fig. 2, text-fig. 8).—The supra-temporal vein enters the postero-dorsal border of the orbital sinus slightly anterior to the anastomosing vein. It runs backwards first as a single stem, but afterwards divides into two in the tissue above the temporalis muscle quite close to the temporal artery and the nervus maxillaris nervus facialis of FISCHER (27). In the posterior part of its course it is sinusoidal in character, and the two vessels are probably connected by anastomosing trunks. It is probably the vessel termed supra-orbitalis by VOGT and JUNG (78), and its opening was noted by WEBER (80), who did not appreciate its significance, thinking it merely a short backward extension of the sinus.

B. XV. *Vena frontalis* (BRUNER) (text-fig. 8).—The frontal vein is formed by the union of anterior and posterior rami, which perforate the frontal bone and unite beneath it near the fronto-parietal suture to form a single vessel. This vein runs laterad of the taenia marginalis (GAUPP, 32) and enters the postero-mesial part of the orbital sinus. It drains the tissues overlying the frontal bone.

B. XVI. *Vena maxillaris* (BRUNER) (text-fig. 8).—The maxillary vein joins the orbital sinus at its postero-lateral corner. It passes outwards and then forwards in the body of the maxilla between it and the jugal; from the substance of the maxilla and its teeth it receives tributaries. Just before it enters the maxilla the labial vein (XVI. β) and the jugal vein (XVI. α) join it. In the region below the nasal capsule it comes out on to the external surface of the maxilla, and it receives in this part of its course two sets of anastomosing vessels, the one putting it into communication with the nasal sinus (B. XIX. α), and the other leading to the anterior network of the lateral palatine sinus (*vide supra*). Just beyond this it receives a small sinus, putting it into communication with the transverse palatine sinus (B. XII. α (i)), and finally it is joined again by the labial vein and immediately runs into the rostral vein (B. XX).

B. XVI. α. *Vena jugalis* (BRUNER) (text-fig. 8).—The jugal vein is a small twig coming from the tissues in the angle of the mouth.

B. XVI. β. *Vena labialis superior* (BRUNER) (text-fig. 8).—As pointed out above, the labial vein joins the maxillary just as it leaves the posterior end of the maxilla, and bending sharply round this bone runs forward in the upper lip. It drains the skin and subcutaneous tissue as it goes along, and rejoins the maxillary as the latter enters the rostral vein.

B. XVII. *Vena nasalis dorsalis* (BRUNER) (text-fig. 8).—The dorsal nasal vein is a small trunk that opens into the antero-mesial region of the orbital sinus. It draws blood from the skin and subcutaneous tissue above the nasal bones through which it passes.

B. XVIII. *Sinus membranæ nictitantis* (BRUNER) (Plate 7, fig. 2, text-fig. 8).—This sinus, which discharges into the anterior part of the orbital just laterad of the orbito-nasal sinus, lies partly in the nictitating membrane and partly in the Harderian gland, into whose substance it penetrates. It also probably receives twigs from the upper eyelid.

B. XIX. *Sinus orbito-nasalis* (Vena orbito-nasalis, GAUPP) (text-fig. 8).—The orbito-nasal sinus is a fairly large sinusoidal vessel, continuing on from the antero-mesial portion of the orbital sinus and draining the large nasal sinus (B. XIX. α), through a large foramen in the orbito-nasal septum. It anastomoses with the maxillary vein in the same region as the latter anastomoses with the lateral palatine sinus.

BRUNER does not describe a similar vessel in *Lacerta* nor in *Tropidonotus*, and, in my own observations (58), I was unable to find this vein in the snake. He states that the nasal sinus discharges by means of a sinus lateralis nasi into the maxillary vein. As just noted, this connection also exists in *Sphenodon*, not as a single vessel, but a series of veins. In *Sphenodon*, however, there is no doubt that the main drainage of the nasal sinus is *viâ* the orbito-nasal sinus. It is not easy to see why there is this difference between *Sphenodon* and *Lacerta*, unless it be that, in the latter form, where the orbit and nasal capsule are relatively further apart, this connection has been lost. It may probably be regarded as more primitive, since it occurs in the Amphibia as the orbito-nasal vein, GAUPP (31), and in *Scyllium*, O'DONOGHUE (59).

B. XIX. α. *Sinus nasalis* (Sinus vestibuli nasi, BRUNER) (text-fig. 8).—The nasal sinus of *Sphenodon* consists of a complex network of anastomosing veins of varying sizes. It ramifies intimately through the peculiar spongy tissue of this region described by LEYDIG (52) and BORN (15) in Lacertilia, who point out its likeness to erectile tissue. In this tissue, in *Sphenodon*, OSAWA (63) first noted the presence of smooth muscle fibres, a point later emphasised by BRUNER (18) in *Lacerta*, and one I am able to confirm. As previously stated, the nasal sinus communicates with the maxillary vein, and apparently with the transverse palatine sinus at its anterior end.

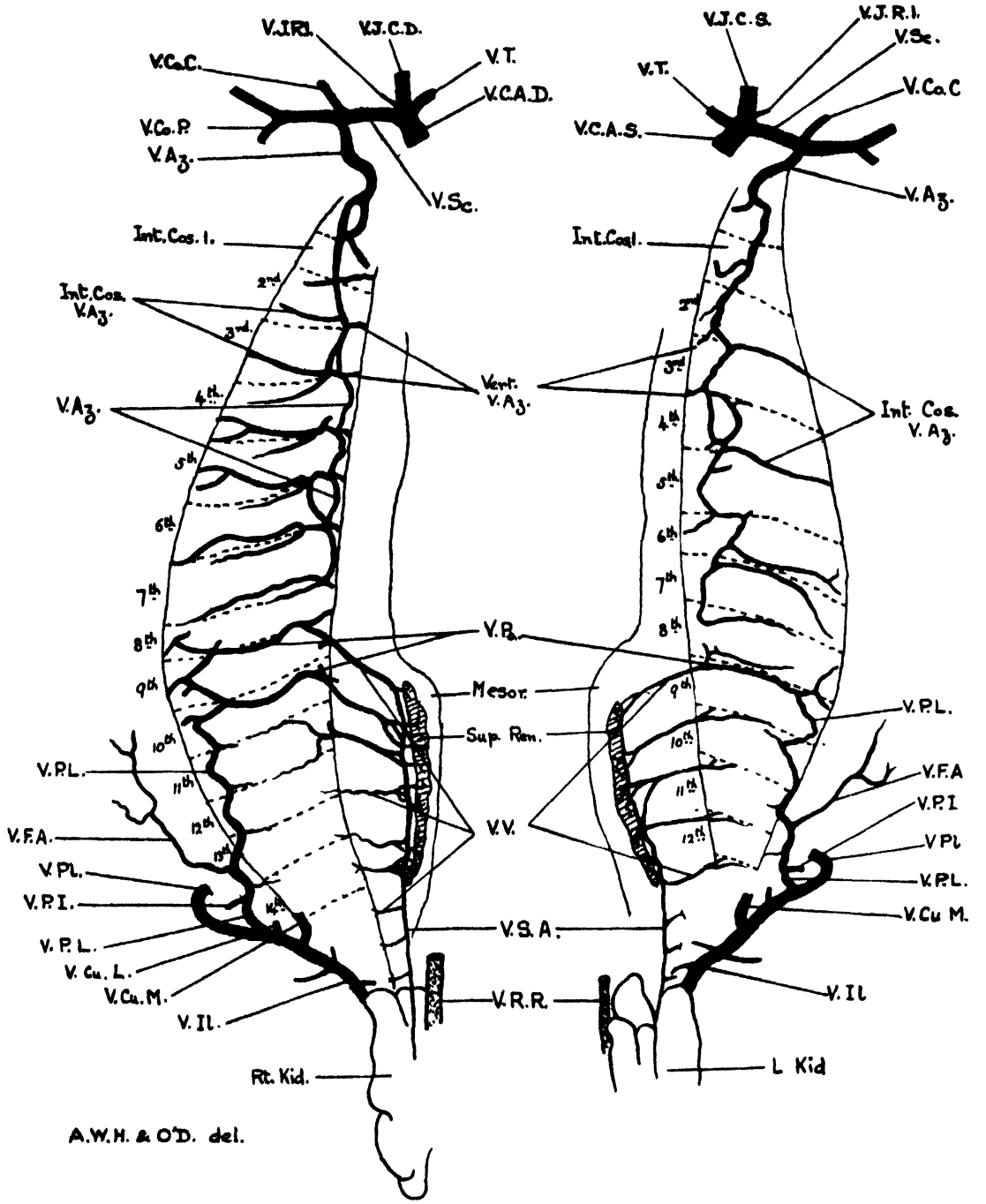
B. XX. *Vena rostralis* (BRUNER) (text-fig. 8).—The rostral vein is a small, irregular transverse vein surrounding the pre-nasal cartilage, and formed by a number of small twigs from the surrounding tissues. At its sides it opens into the maxillary veins in the manner already described, and so not only places them in communication with each other, but also is itself connected through them with the labial vein and transverse palatine sinus.

C. *Vena subclavia* (CORTI) (Plate 6, fig. 1; Plate 7, fig. 2, text-figs. 7 and 9).—The subclavian is the third of the great anterior veins that unite to form the

pre-caval vein. It drains the fore limb, and, in addition, receives factors from the shoulder girdle and the ventral and dorsal body wall. Very little is known about the veins of the limbs in the Lacertilia, save a quite brief account of *Uromastix spinipes* given by HOCHSTETTER (46), with which the present account does not entirely agree, and I know of no reference to them in *Sphenodon*. They differ considerably from *Rana*, where, as has been fully described by GAUPP (31), two large venous trunks come from the upper arm, the brachial and the subclavian, so that it is hard to draw homologies between the two animals, and, as might be expected, *Sphenodon* differs considerably from the mammal. The subclavian is a large trunk that runs straight outwards from its point of union with the jugular and tracheal. A short distance along it receives on its anterior wall a factor (C. I) from the pectoral girdle, and the Azygos (C. II) enters it on the opposite side. About an equal distance further out it receives posteriorly another well-marked factor from the pectoral girdle (C. III), after which it may be termed the Vena axillaris. Just before entering the limb, the great cutaneous vein (C. IV) runs into it on the antero-dorsal side, and the main trunk, passing into the arm, becomes the vena brachialis. On its way to the elbow, along the superficial internal side of the upper arm, three large tributaries (C. V–VII) join it, and, after passing that joint, it becomes the vena antibrachialis superficialis of GAUPP. This passes slowly across the extensor side of the fore arm to its radial border, again receiving three well-marked branches (C. VIII–X), and, at the base of the hallux, turns mesiad, to form the arcus venosus dorsi manus. The arcus first gives off a vessel (C. XI) that anastomoses with its own distal extremity on the ulnar side, and then a series of venæ interdigitales (C. XII) to the spaces between the digits. Finally, it runs round on to the flexor side of the fore arm, and a short way up towards the elbow again, along the outer border of the "Anconæus quartus (richtiger quintus)" muscle of OSAWA.

C. I. *Vena coraco-clavicularis* (text-fig. 9).—The coraco-clavicular vein enters the anterior wall of the subclavian about 1 cm. before it flows into the anterior vena cava. It drains the dorsal part of the shoulder girdle, and passes ventrally between clavicle and coracoid and between the costo-scapularis and costo-coracoideus muscles. It receives a factor from the clavicular region.

C. II. *Vena azygos* (BEDDARD; V. vertebralis posterior, HOCHSTETTER) (text-fig. 9).—The azygos is a large interesting vein, entering the posterior wall of the sub-clavian nearly or quite opposite the coraco-clavicular. It can be traced backwards on both sides of the body to the posterior border of the seventh intercostal space. The factors joining it come from both the costal and vertebral regions, so that the territory it drains corresponds with that



TEXT-FIG. 9.—Dissection of Azygos, Iliac, Afferent Supra-Renal and Associated Veins on both sides.

Veins.—*Int.Cos.Az.*, intercostal branches of vena azygos; *V.Az.*, vena azygos; *V.C.A.D.*, vena cava anterior dextra; *V.C.A.S.*, vena cava anterior sinistra; *V.Co.C.*, vena coraco-clavicularis; *V.Co.P.*, vena coraco-pectoralis; *V.Cu.L.*, vena cutanea lateralis; *V.Cu.M.*, vena cutanea magna; *Vert.V.Az.*, vertebral tributaries of vena azygos; *V.F.A.*, vena flexoris abdominalis; *V.J.C.D.*, vena jugularis communis dextra; *V.J.C.S.*, vena jugularis communis sinistra; *V.J.R.I.*, ramus muscularis of vena jugularis communis; *V.Pa.*, venæ parietales; *V.P.I.*, vena pubo-ischio-trochanterica; *V.Pl.*, vena pelvica; *V.P.L.*, vena parietalis lateralis; *V.R.R.*, venæ renales revehentes; *V.S.A.*, vena supra-renalis advehens; *V.Sc.*, vena subclavia; *V.T.*, vena trachealis; *V.V.*, vena vertebralis.

*Int.Cos.*1., first intercostal space; 2nd–14th, second, &c., intercostal spaces; *L.Kid.*, left kidney; *Mesor.*, mesorchium; *Rt.Kid.*, right kidney; *Sup.Ren.*, dorsal surface of supra-renal body.

of the posterior cardinal of the embryo, and there is little doubt it is the actual remains of the anterior part of this. The posterior part of the embryonic post-cardinal probably persists as the afferent supra-renal, as will be discussed later. The break between the two is a very slight one in *Sphenodon*, for the factors of the latter vessel commence in the eighth intercostal space. There is, as far as can be seen, no actual connection between them such as HOCHSTETTER states he has found in Lacerta (46), and furthermore, I have not been able to find one or more venæ hepaticæ advehentes running from the azygos to the portal veins such as are described by the same author.

One of the earliest descriptions of this vessel in Lacertilia we owe to JOURDAIN (49), who describes it as "étendue depuis la jugulaire antérieure jusqu'à la queue," a description that is hardly accurate. According to PARKER (64), it is present only on the right side in *Lacerta viridis*, and this is perhaps substantiated by HOCHSTETTER (*loc cit.*, p. 457), who terms it the vena vertebralis posterior, and says: "Das Blut aus der linkseitigen V. vertebralis posterior wird durch eine in dieser Gegend stärker entwickelte Queranastomose, von der bereits oben die Rede war, der V. vertebralis dextra zugeführt." The description of this author is not clear, however, for on the same page he apparently calls the vessel the A. vertebralis posterior and seems to suggest it is present on both sides.

Considerable variation in the presence and size of this vessel and the extent of its development on the two sides of the body is to be encountered in the Lacertilia, as has been shown mainly by BEDDARD. It may be present on both sides, and then, as a rule, one is considerably larger than the other. The better developed one may be on the left, as in *Varanus griseus* (8), *Iguana tuberculata* (2), and *Chamæleo sp.* (4), or on the right, as in *Pygopus lepidopus* (4). In yet other cases the vein is absent altogether on one side; the right in *Ophisaurus apus* (*Pseudopus pallasii*) (7); the left in *Phelsuma madagascariensis*, *Tarentola annularis* (4), and in *Heloderma suspectum* (8). The condition in *Sphenodon* is of interest because it is a primitive one, from which that found in any of the Lacertilia can be derived. In the first place, the two vessels are much more nearly of a size than in other forms, and, in the second, they extend back farther than most species, even where the two vessels are present in a fairly marked manner.

C. III. *Vena coraco-pectoralis* (V. thoracica anterior? HOCHSTETTER) (text-fig. 9).—This vessel comes from the ventral portion of the shoulder girdle and enters the sub-clavian vein laterad of the Azygos. The main trunk, which perhaps is homologous with the anterior thoracic of HOCHSTETTER, although it is not the only vessel coming from the girdle, may be regarded as the one passing out to drain the dorsal surface of the coracoideus and adjacent

muscles. It receive two tributaries, one from the pectoralis muscle (C. III. α), and one from the sterno-coracoideus (C. III. β).

C. III. α. *Ramus pectoralis.*—The pectoral branch drains the dorsal surface of the pectoralis muscle and also picks up a well marked tributary from the ventral surface of the same.

C. III. β. *Ramus sterno-coracoideus.*—The sterno-coracoid branch comes from the internus superficialis and internus profundus portions of the similarly named muscle.

The main trunk may now be termed the *Vena axillaris*, as it accompanies the axillaris nerve.

C. IV. *Vena cutanea magna* (BETHGE) (Plate 8, fig. 8).—This vein enters the dorso-lateral extremity of the vena axillaris immediately before that vessel becomes the branchial vein. It comes in through the dorsal shoulder muscles from the external surface of the latissimus dorsi muscle, where it receives a small anterior tributary (C. IV. α). The main trunk passes backwards in the latero-dorsal subcutaneous tissue to the region of the pelvis, where it again dives through the musculature to enter the pelvic vein, thus forming a factor (A. III) of the system of the anterior abdominal vein (*q.v.*). This posterior connection is not quite the same as that described by BETHGE (11) in *Salamandra* and *Triton*, but the vessel appears from its general relation and distribution to be the same, so that I have adopted BETHGE'S term of great cutaneous vein. It is not improbable that it will prove to be also homologous with the lateral cutaneous vein of the Elasmobranch (59).

C. IV. α. *Vena cutanea parva* (BETHGE).—The small cutaneous vein joins the great vein in the subcutaneous tissue dorsad of the latissimus dorsi muscle. It drains the tissue in the anterior continuation of the same line as the great vein and can be traced forwards to just behind the head.

The main trunk now enters the posterior aspect of the upper arm and so may be termed the *Vena brachialis* (Plate 8, figs. 7 and 8).

C. V. *Vena profunda brachii* (GAUPP) (Plate 8, figs. 7 and 9).—This is obviously not quite the same vessel as the profunda brachii of the frog, since in this animal the vein is a continuation of the subscapular, but as it has a very similar distribution it appears to be justifiable to give it the same name. It drains the cleido-humeralis, supra-coracoideus, anconæus and adjacent muscles, and receives near its end the Vena anastomotica longa (C. X).

C. VI. *Ramus muscularis* 1.—This branch runs into the radial side of the brachial vein just at the elbow, and comes mainly from the ends of the supinator and extensor digitorum communis longus muscles.

C. VII. *Ramus muscularis* 2.—This is the second muscular branch at the elbow, and it passes outwards superficially to the distal end of the anconæus and neighbouring muscles.

The main trunk now passes into the fore arm as the *Vena antibrachialis superficialis* of GAUPP, and is obviously the same vessel as that described by HOCHSTETTER (46) in *Uromastix* as "Eine veine im Sulcus radialis antibrachii" (Plate 8, figs. 7 and 9).

C. VIII. *Vena interossea* (GAUPP) (Plate 8, figs. 7 and 9).—The interosseal vein, probably the "Ulnare Randvene" of HOCHSTETTER, is a fair sized trunk coming off the superficial antibrachial, passing round on to the flexor surface of the proximal end of the radius and over towards the ulnar side of the limb. It runs down deep in the muscles, draining the flexor carpi ulnaris and pronator quadratus, into the hand. Unfortunately it could not be followed satisfactorily, but it appears to receive certain interdigital volar veins and to be connected with the main vessel on the extensor side by anastomoses, one of which is in the region of the bone of the hallux (*vide infra*).

C. IX. *Vena præhallucis* (GAUPP) (Plate 8, fig. 7).—This vein leaves the superficial antibrachial near the carpus and passes to the outside of the thumb, where it probably anastomoses with the veins on the palmar surface of the hand as just noted.

C. X. *Vena anastomotica longa* (Plate 8, fig. 7).—This long anastomosing vein comes off from the main vessel in the region of the distal end of the extensor carpi radialis longus muscle and runs right back up the fore arm and upper arm, following a course roughly parallel with that of the main trunk, but quite superficial. It flows into the brachial vein *vid* the end of the vena profounda brachii (C.V) near the proximal end of the anconæus.

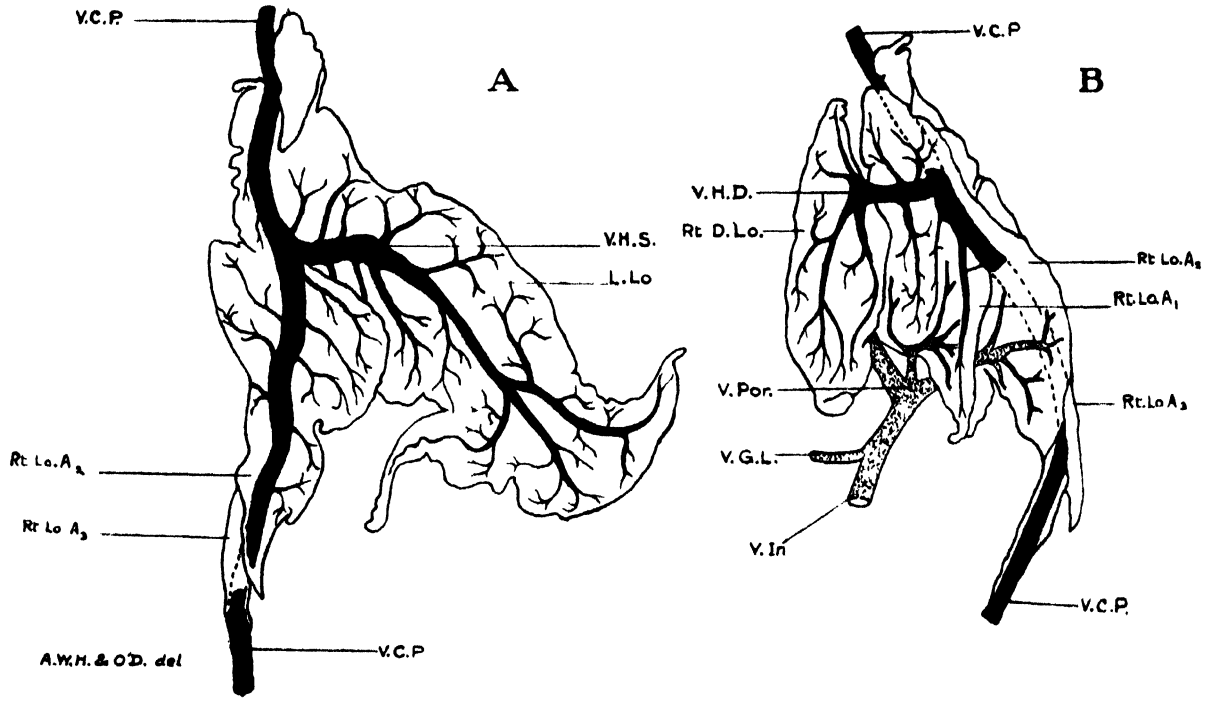
The main trunk now begins to turn across to the ulnar side of the hand, forming a well marked *arcus venosus dorsi manus.*

C. XI. *Vena anastomotica arcus venosi* (Plate 8, fig. 7).—This anastomosing branch comes off from the arcus venosus on the radial side and runs over the back of the hand, to enter the arcus again on its ulnar side.

C. XII. *Venæ interdigitales* (Plate 8, fig. 7).—Four well-marked interdigital veins are received by the arcus, each formed by the union of two lateral digital veins at the base of the space between each respective pair of digits. The fifth vein, corresponding to these, drains the external side of the fifth digit.

Vena cava posterior (Plate 6, fig. 1, text-figs. 10 and 11).—The post-caval vein is a large thin-walled trunk lying in the dorsal mesentery slightly to the right of the

middle line and continuing in the line of the right efferent renal vein. It is formed by the union of two efferent renals (E) at the level of the posterior end of the left testis and passes forwards to the attenuated extremity of the right lobe of the liver. Partly embedded in this organ the vessel runs forward to its anterior end, receiving on its way a number of small hepatic veins and two much larger ones, a right (A) and a left (B). In front of the liver the post-caval vein passes freely forward in the mesentery to enter the posterior end of the sinus venosus. Posterior to the liver it receives the right spermatic vein (C), while the left spermatic vein (D) opens into the short anastomosis running transversely between the ends of the efferent renal veins.



TEXT-FIG. 10.—(*a*) Ventral aspect of Liver to show Post-Caval and Left Hepatic Veins. (*b*) Right side of Liver to show Post-Caval, Right Hepatic and Hepatic Portal Veins. The junction of the anterior gastric and anterior abdominal veins with the hepatic portal is hidden by the lobes of the liver.

Veins.—*V.C.P.*, vena cava posterior; *V.G.L.*, vena gastero-lienalis; *V.H.D.*, vena hepatica dextra; *V.H.S.*, vena hepatica sinistra; *V.In.*, vena intestinalis; *V.Por.*, vena portæ.

Rt.D.Lo., right dorsal lobule of liver; *Rt.Lo.*, A 1, A 2, and A 3, the three right ventral lobules of liver; *L.Lo.*, left lobe of liver.

A. *Vena hepatica dextra* (text-fig. 10).—The right hepatic vein is a fair-sized vessel composed of a number of factors from the various lobes of the liver on the right side. It enters the post caval about 3·5 cm. behind the point where this vessel leaves the anterior end of the liver.

B. *Vena hepatica sinistra* (text-fig. 10).—The left hepatic vein is smaller but very similar to the right, save that it comes from the left lobes of the liver and enters the left wall of the post caval just caudal to the point of entry of the right hepatic vein.

C. *Vena spermatica dextra* (Plate 6, fig. 1, text-fig. 11).—The right spermatic vein arises as a network of small vessels in the mesorchium at the anterior end of the testis. It then runs backwards along the supra-renal bodies, which it almost covers, receiving the efferent supra-renal veins (C. I), and also receiving as a rule four fair-sized veins from the testis. At the posterior end of the testis it turns sharply inwards and flows into the post-caval vein.

C. I. *Venæ supra-renales revehentes.*—These are a series of minute twigs coming from the supra-renal body, opening into the spermatic vein during its course along that gland and serving as efferent supra-renal veins.

D. *Vena spermatica sinistra* (Plate 6, fig. 1, text-fig. 11).—The relations of the left spermatic vein to the corresponding supra-renal body and testis are similar to those on the right, save that, as a rule, it has five branches from the testis instead of four. It does not open directly into the post-caval vein, but into the transverse anastomosis joining the left efferent renal to the right.

D. I. *Venæ supra-renales revehentes.*—A series of efferent supra-renal veins, similar to those on the right, open into the left spermatic vein.

E. *Venæ renales revehentes* (HOCHSTETTER) (Plate 6, fig. 1, text-fig. 11).—The efferent renal veins commence in the substance of the kidneys at their posterior ends and run forward as fair-sized thin-walled vessels on the median borders of their ventral faces and separated from one another in the middle line by the dorsal mesentery. The right vein, from the start, is much larger than the left, a condition that is common in the Lacertilia (*vide* BEDDARD, 7), and the two are joined by four large inter-renal anastomoses. Herein *Sphenodon* differs from both *Lacerta viridis*, *L. ocellata*, and *Varanus arenarius*, in which, according to HOCHSTETTER (46), there is only one small anastomosis at the posterior end of the kidneys. Between the kidneys and the place where they join one another, both veins receive a number of small tributaries from the mesentery. As already stated, the left efferent renal pours its blood into the right at the level of the hinder end of the left testis, by means of a short stout anastomosis into which the left spermatic vein runs.

The remaining veins of the body are not concerned with taking the blood back directly to the heart, and so can be dealt with conveniently as a series of distinct but often closely related systems.

The Supra-renal Portal System.

The presence of this system has already been noted in the *Varanidæ* by CORTI (21), HOCHSTETTER (46), and BEDDARD (8), in which genus it is very well developed. It is subject to a great deal of variation in the Lacertilia, as BEDDARD has shown, and, although usually present and often well marked, as in the *Varanidæ*, *Iguana*

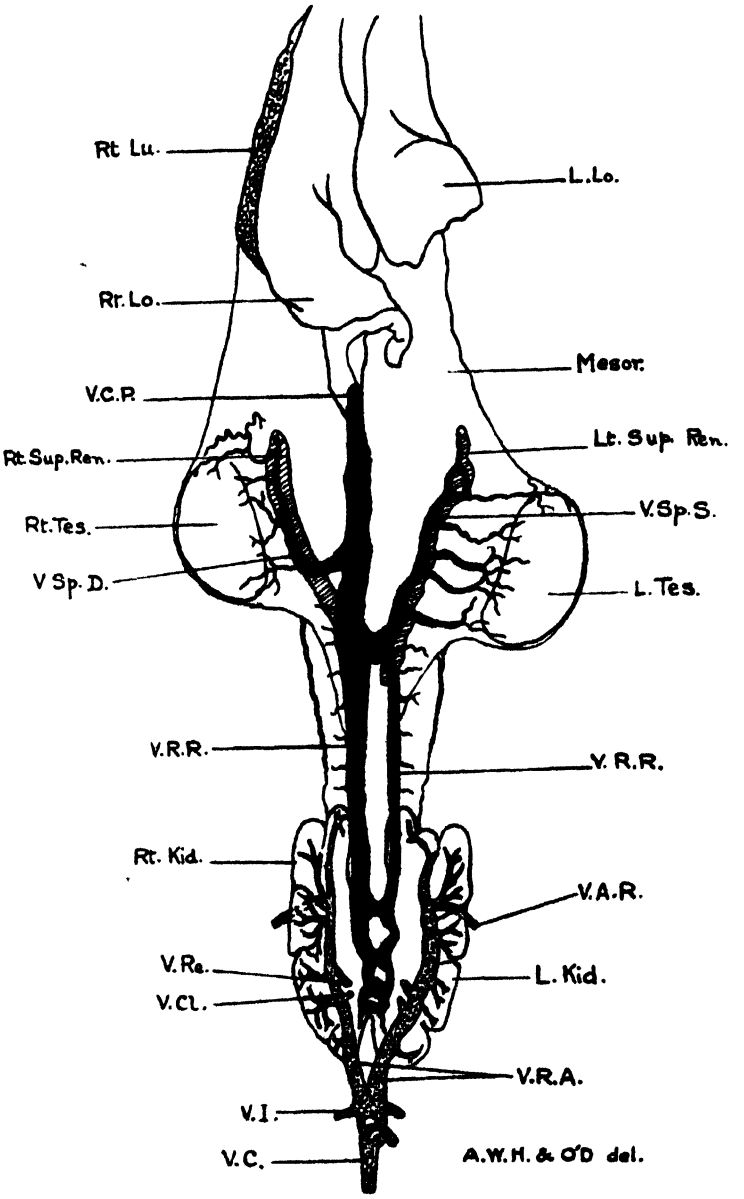
tuberculata (2), *Ophisaurus apus* and *Amphisbæna brasiliana* (7),* it may be considerably reduced, *e.g.*, *Tiliqua scincoides* (2), or even absent altogether, save as an abnormal variation, as in *Chamæleon vulgaris* (4). In *Sphenodon* it has apparently retained more primitive relations than in the other forms described, and this throws useful light on the question of its constitution. Its main factor is a vessel, termed by HOCHSTETTER (46) the Vena deferentialis, since it accompanies the vas deferens, and by BEDDARD (7) the post cardinal vein. With regard to this vessel, BEDDARD, after pointing out that it receives branches from the parietes, adds: "Considering . . . the relation of the vein to the vas deferens (Wolffian duct), I imagine that it is to be regarded as a persistent, though small, posterior cardinal vein." The position of the vessel in *Sphenodon*, I think, fully supports this interpretation. The azygos vein, Vena vertebralis of HOCHSTETTER, is generally admitted to be a persistent part of the embryonic posterior cardinal vein, and it receives factors not only from the parietes, but also from the vertebral region. It will be seen that the vein in question similarly has parietal and vertebral branches at its anterior end, and, indeed, the break between it and the azygos on both sides is very small, being only from one vertebra to the next. In *Lacerta viridis*, as figured by HOCHSTETTER, it would appear as if there is not even this hiatus. Moreover, the posterior end of the vein disappears in the kidney substance as the old posterior cardinal would. Then again, in *Varanus arenarius*, HOCHSTETTER (*loc. cit.*, Plate 16, fig. 16) depicts the vein as opening into the anterior end of the afferent renal, and so directly continuous with the caudal vein. There seems to be little doubt, then, that this vessel is actually a persistent, though much reduced, portion of the posterior cardinal vein.

A. *Vena supra-renalis advehens* (V. cardinalis posterior, BEDDARD; V. deferentialis, HOCHSTETTER) (text-fig. 9).—The term afferent supra-renal vein seems preferable to posterior cardinal, as employed by BEDDARD, since the vessel is only a small part of the embryonic posterior cardinal, while the azygos is a larger persistent portion of the same trunk, and so more deserving of the name. The term vena deferentialis of HOCHSTETTER is open to the objection that it is obviously unsuitable in the case of the female. Afferent supra-renal is free from both these objections, and at the same time indicates its anatomical relation to the supra-renal gland by pairing it with the efferent supra-renal veins. In *Sphenodon* the vessel arises in the anterior end of the kidney, and passes forwards alongside the vas deferens to the anterior end of the supra-renal body to the substance, of which it gives off a large number of small twigs. Along its course it receives factors from the vertebral region (A. I), and also from the parietes (A. II).

* This vessel does not appear to be present in a female *Amphisbæna cinerea* examined by V. BEDRIAGA (9). He figures the parietal veins from this region of the body as opening directly into the efferent renal veins.

A. I. *Venæ vertebrales* (text-fig. 9).—The vertebral veins come from the region of the vertebræ. There are nine of them on the right side and eight on the left, opening into the afferent supra-renal vein, and the anterior vessels on each side are connected with the corresponding parietals.

A. II. *Venæ parietales* (text-fig. 9).—On the right side are two large parietal veins which unite with their corresponding vertebrals, and, by means of the common trunk so formed, drain into the afferent supra-renal. At their



TEXT-FIG. 11.—The Post Caval, Renal Portal, Renal Efferent and Spermatic Veins, and their connections.

Veins.—*V.A.R.*, vena anastomotica renalis; *V.C.*, vena caudalis; *V.Cl.*, vena cloacalis; *V.C.P.*, vena cava posterior; *V.I.*, vena inguinalis; *V.R.A.*, venæ renales advehentes; *V.Re.*, vena rectalis; *V.R.R.*, venæ renales revehentes; *V.Sp.D.*, vena spermatica dextra; *V.Sp.S.*, vena spermatica sinistra.

L.Kid., left kidney; *L.Lo.*, left lobe of liver; *Lt.Sup.Ren.*, left supra-renal body; *L.Tes.*, left testis; *Mesor.*, mesorchium; *Rt.Kid.*, right kidney; *Rt.Lo.*, right lobes of liver; *Rt.Lu.*, right lung; *Rt.Sup.Ren.*, right supra-renal body; *Rt.Tes.*, right testis.

distal extremity these two parietal veins open into the lateral parietal vein, so forming well marked anastomoses between it and the afferent supra-renal, placing the supra-renal portal system into communication with the system of the caudal vein. On the left side there is only one similarly constituted vein.

The next two right parietal veins are reduced, and join together before uniting with the vertebral. The parietal factors on both sides caudad of those described get so much reduced that they practically disappear.

The System of the Caudal Vein (text-fig. 11).

This system forms in the main a portal system to the kidneys, *i.e.*, most of the blood brought by it passes through the substance of the kidneys, and is returned to the heart by way of the efferent renal veins.

A. *Vena caudalis* (text-fig. 11).—The caudal vein commences far back in the tail, and runs forward in the hæmal arches, receiving paired factors from the muscle segments on its way. Just before reaching the hinder end of the kidneys it becomes swollen, receives veins from the inguinal region (A. I), and shows a tendency to split into two. Finally, it does divide into two veins, which diverge and enter the posterior ends of the kidneys, becoming the afferent renal veins (B).

A. I. *Vena inguinalis* (text-fig. 11).—The inguinal vein is a well marked vessel on each side bringing in blood from the inguinal region.

B. *Venæ renales advehentes* (text-figs. 11 and 13).—The right afferent renal or Jacobson's vein is generally smaller than the left. They both enter the posterior ends of the corresponding kidneys and run forward, partially buried in the kidney substance in its mid-ventral line. Each receives a cloacal (B. I) and a rectal factor (B. II). The vessel gradually diminishes in size, breaking up in the kidney. It sends a fair sized branch in the deep cleft between the first and second kidney lobes which turns dorsally and enters the iliac vein, thus establishing a direct connection between the systems of the caudal and abdominal veins. BEDDARD (7, p. 464) states that in *Sphenodon* he was unable to find any such connection, and opines that if the two systems are connected at all it is only indirectly through the kidney substance. The vessel, although hidden in a cleft, shows clearly in a well injected specimen, and was also found in the other specimens examined. It is a vein very similar to that figured in *Lacerta* by HOCHSTETTER (46, Plate 16, fig. 12), save that it runs in a cleft in the kidney and not superficially across its ventral surface.

B. I. *Vena cloacalis.*—The cloacal vein is a small tributary from the side of the cloaca entering the afferent renal about 6–8 mm. from the posterior end of the kidney.

B. II. *Vena rectalis.*—The rectal vein comes from the dorsum of the anal gland and receives small factors from the base of the bladder. It enters the afferent renal just anterior to the cloacal vein.

The following three systems are, strictly speaking, all parts of the one hepatic portal series of veins, since they all take blood to the liver. For convenience, however, they may be treated separately and dealt with as a main hepatic portal system with two closely related accessory systems, that of the anterior abdominal vein and that of the epigastric veins.

The Hepatic Portal System (text-figs. 10 and 12).

This system is, on the whole, similar to that in *Lacerta viridis* and figured by HOCHSTETTER (*loc. cit.*, Plate 16, fig. 15). It differs mainly in the fact that it has a large anterior gastric factor, which itself gives off a number of portal branches directly to the liver.

A. *Vena porta* or *Vena hepatica advehens* (HOCHSTETTER) (text-figs. 10 and 12).—The hepatic portal vein is quite a short trunk formed mainly by the union of the lieno-gastric (A. I) and intestinal (A. II) veins, and is distributed mainly to the right lobes of the liver, while the left lobes of the liver are supplied almost entirely by the anterior gastric factor (A. III).

A. I. *Vena gastero-lienalis* (HOCHSTETTER) (text-fig. 10).—The lieno-gastric vein is formed at the anterior end of the spleen, when a number of small factors and one larger one (A. I. β) from that body join a small gastric vein (A. I. α). It is soon joined by another small gastric tributary (A. I. γ) and runs in the mesentery alongside the pancreas, from which it receives a vessel (A. I. δ). Finally it joins the intestinal vein in the neighbourhood of the pylorus, from which part of the gut it also receives blood (A. I. ϵ).

A. I. α. *Vena gastrica* 1.—The first gastric vein is a small vessel arising from the left hand side of the stomach about half-way down. It runs freely in the lieno-gastric omentum, and at the anterior end of the spleen receives from that body six or eight small veins.

A. I. β. *Vena lienalis.*—The splenic vein is a trunk formed by the union of a number of small factors from the posterior end of the spleen, and after a short course it unites with the first gastric vein to form the lieno-gastric.

A. I. γ. *Vena gastrica* 2.—The second gastric vein is another small vessel coming from the caudal end of the cardiac part of the stomach and entering the lieno-gastric shortly after this leaves the spleen.

A. I. δ. *Venæ pancreaticæ.*—As the main trunk passes the pancreas a number of small tributaries from that gland flow into it.

A. I. ϵ. *Vena pylorica.*—The pyloric vein is constituted by the union of a

number of a small branches from the region of the pylorus, and it joins the main trunk just before it enters the intestinal.

A. II. *Vena intestinalis* (text-fig. 10).—The intestinal vein commences low down on the rectum as a rectal vein, and leaving its cranial end runs forward in the mesentery to unite with the lieno-gastric. During its path through the mesentery two tributaries from the intestine (A. II. β and γ) and one from the duodenum (A. II. α) flow into it.

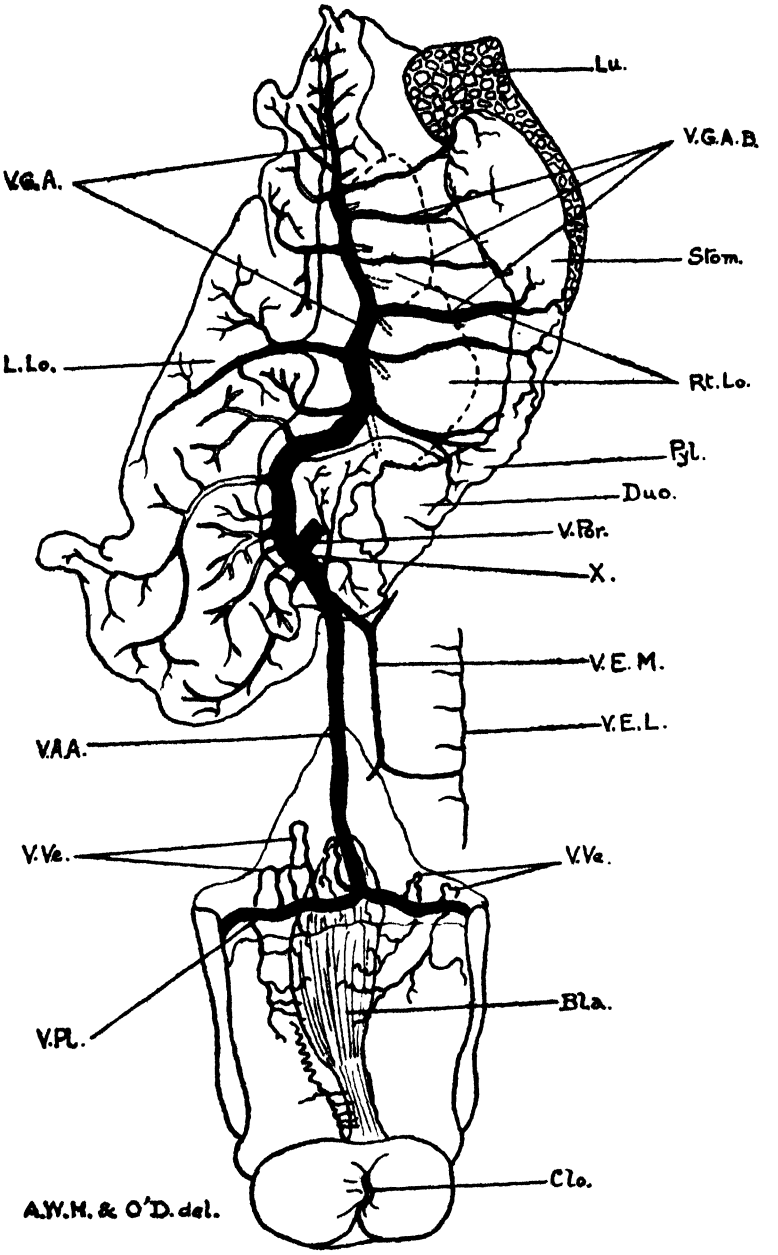
A. II. α. *Vena duodenalis.*—The duodenal vein is composed of several branches from the duodenum, which unite to form a single vessel entering the intestinal vein just before it joins the lieno-gastric.

A. II. β. *Vena intestinalis anterior.*—The front end of the intestine is drained by the anterior intestinal vein, which enters the main intestinal vein as it passes through the mesentery.

A. II. γ. *Vena intestinalis posterior.*—The posterior intestinal vein comes from the remaining and hinder part of the intestine as far back as the rectum. It reaches the main trunk a short distance caudal of the anterior intestinal vein.

A. III. *Vena gastrica anterior* (text-fig. 12).—The anterior gastric vein is a large vessel originating in the gastro-hepatic omentum near the anterior end of the stomach in a twig coming from that organ. It passes backwards close to the left lobe of the liver, receiving five or six large tributaries coming from the whole of the stomach back to the duodenum. Near the end of that lobe of the liver it bends in suddenly to enter the main hepatic portal trunk as this enters the right liver lobes. Just at the point where it turns it receives the large anterior abdominal vein (A. III. α); indeed, it appears as if it were a continuation of that vessel, as a glance at the text-figure (*i.e.*, 12) will show.

BEDDARD, indeed (7, p. 464), regards it as a forward extension of that vein, for he says: "The anterior abdominal vein, reinforced by the portal, runs in the membrane which connects the stomach with the left lobe of the liver, giving off branches to the liver substance and receiving at intervals branches from the stomach. Towards the anterior end of the liver the conjoined porto-abdominal trunk finally disappears in the liver." He instances an example of a similar distribution in a snake, *Eryx* (5), and claims *Pygopus* as showing an intermediate condition (7). In spite of its appearance, however, I think it better to regard this vessel as an anterior gastric factor of the hepatic portal system corresponding to the several gastric veins in *Lacerta viridis* according to HOCHSTETTER (2), which open separately into the liver. The vessel, moreover, is in a similar position to a corresponding gastro-hepatic factor in *Salamandra* and *Triton*, with which it is no doubt homologous. The matter can only be definitely settled, however, by reference to its development, but, of course, there must be some such portal vessel or vessels draining the



TEXT-FIG. 12.—Diagram of the Anterior Abdominal Vein, showing its relation to the Liver, the Epigastric and Hepatic-Portal Veins.

Veins.—*V.A.A.*, vena abdominalis anterior; *V.E.L.*, vena epigastrica lateralis; *V.E.M.*, vena epigastrica media; *V.G.A.*, vena gastrica anterior; *V.G.A.B.*, gastric branches of the vena gastrica anterior; *V.Pl.*, vena pelvica; *V.Por.*, vena porta; *X.*, point of entrance of the anterior abdominal vein into the anterior gastric as the latter joins the main hepatic portal trunk.

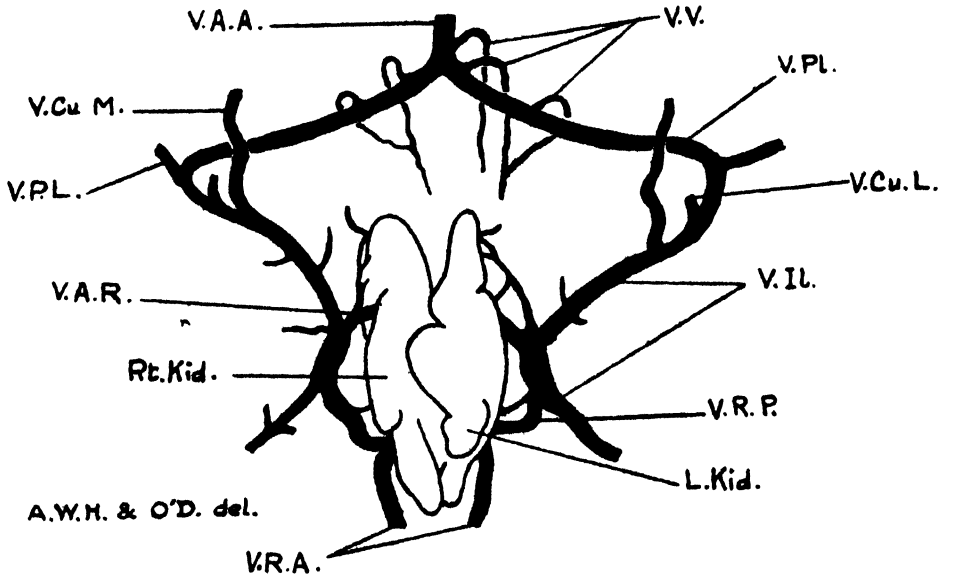
Bla., bladder; *Clo.*, cloaca; *Duo.*, duodenum; *L.Lo.*, left lobe of liver dorsal surface; *Lu.*, lung; *Pyl.*, pylorus; *Rt.Lo.*, right lobes of liver seen through mesentery; *Stom.*, stomach.

stomach before the two lateral abdominal veins have joined to form the single anterior abdominal.

All along its course it gives numerous short venæ advehentes, mostly to the left lobes of the liver, but some also to the right, so that most of its blood

can go straight to the liver substance without entering the main hepatic portal trunk. Indeed, as will be seen, most of the blood from the main portal vessel goes to the right lobes, while the bulk of that from the anterior abdominal vein and the anterior gastric goes to the left lobes.

A. III. *α. Vena abdominalis anterior.*—The anterior abdominal vein, although really only a factor of the anterior gastric, is here treated separately as a matter of convenience.



TEXT-FIG. 13.—The Pelvic Ring, showing the relation of the Iliac, Renal, and Anterior Abdominal Veins. The outer edges of the kidney turned over mesially.

Veins.—*V.A.A.*, vena abdominalis anterior; *V.A.R.*, vena anastomotica renalis; *V.Cu.L.*, vena cutanea lateralis; *V.Cu.M.*, vena cutanea magna; *V.Il.*, vena iliaca communis; *V.Pl.*, vena pelvica; *V.P.L.*, vena parietalis lateralis; *V.R.A.*, venæ renales advehentes; *V.R.P.*, vena renalis posterior; *V.V.*, venæ vesicæ.

L.Kid., left kidney; *Rt.Kid.*, right kidney.

The System of the Anterior Abdominal Vein (text-figs. 12 and 13).—As has just been pointed out, the anterior abdominal vein opens into the anterior gastric branch of the hepatic portal vein, and so is portal to the liver.

Vena abdominalis anterior (HOCHSTETTER) (text-figs. 12 and 13).—The anterior abdominal vein is formed in the mid-ventral line by the union of the two pelvic veins (A), which, in their turn, are continuations of large branches from the common iliac veins (B). Its relations to the common iliac veins and to the afferent renal system is very similar to that in *Salamandra maculosa* as figured and described by HOCHSTETTER (45), and also in *Salamandra*, *Triton* and *Spelerpes* according to BETHGE (11). Although there seems to be a connection between the roots of the anterior abdominal and afferent renal vessels generally in Lacertilia, it varies considerably in different forms, as has been pointed out by HOCHSTETTER (46) and in BEDDARD'S useful series of papers (2–5). It is interesting to find that *Sphenodon*

and *Lacerta* retain the primitive Urodele condition. Just before entering the liver it may receive the epigastric vein (C).

A. *Vena pelvica* (Wurzel der V. abdominalis, HOCHSTETTER; R. abdominalis, GAUPP) (text-figs. 12 and 13).—The pelvic veins may be considered as arising where the common iliac veins anastomose with the afferent renal system, although they are to all appearances direct continuations of the common iliac veins. They pass forward laterally, and then turn in rather sharply ventralwards to unite in the mid-ventral line and give origin to the anterior abdominal vein. During their course each receives a series of factors (A. I–VI).

A. I and II. *Venæ parietales.*—Just as the pelvic vein turns laterally it receives a couple of small factors from the posterior parietes.

A. III. *Vena cutanea magna* (BETHGE) (text-figs. 9 and 13).—The great cutaneous vein arises from the pelvic just in front of the point where this vessel crosses the femoral nerve. It pierces the muscles and passes forwards in the subcutaneous tissue of the latero-dorsal body wall to the scapular region. Here, again, as pointed out above, it leaves its subcutaneous position, and piercing the muscles, flows into the axillaris vein laterally to the azygos vein. In spite of the fact that its posterior connections are somewhat different, it is no doubt homologous with the V. cutanea magna of the Urodela *Salamandra*, *Triton*, etc., as described by BETHGE (11).

A. IV. *Vena cutanea lateralis* (text-figs. 9 and 13).—The lateral cutaneous vein is a smaller but nevertheless well marked vein coming from the skin of the lateral body wall. It joins the pelvic shortly after the great cutaneous vein.

A. V. *Vena parietalis lateralis* (Lateral abdominal, BEDDARD) (text-figs. 9 and 13).—This vein is described by BEDDARD (*loc. cit.*) in a number of Lacertilian forms and appears to be a constant factor of the pelvics, although exhibiting a varying degree of development in different species. It has been termed the lateral abdominal vein, but this term seems open to exception.

In the Elasmobranchs (59) the lateral abdominal vein is a more ventral vessel, running from the iliac forward to the subclavian vein latero-ventrally under the peritoneum on each side. According to MARSHALL (56) in the Tadpole and GOETTE in *Bombinator igneus* (35), these two lateral veins are fused to form the median anterior abdominal vein, which acquires a secondary connection with the hepatic portal system. Certain abnormal adult specimens of *Rana*, BULLER (19), WOODLAND (81), and O'DONOGHUE (57), seem to bear out this account. If, then, this description of the formation of the anterior abdominal vein be correct, the vessel now to be described is not the homologue of the lateral abdominal of the fish. The term lateral parietal seems to be suitable, since in *Sphenodon* it arises at the level of the eighth inter-

costal space from a parietal vein which connects it with the supra-renal portal system (*q.v.*).

It passes backwards in the angle between dorsal and ventral body wall, receiving small parietal factors from the intercostal spaces. Before entering the pelvic vein it receives two tributaries from the adjacent musculature (A. V. α and β).

A. V. α. *Vena flexoris abdominis.*—This is a moderate sized vessel, flowing into the lateral parietal vein at the level of the hinder end of the thirteenth intercostal space. It drains the flexor abdominis muscle.

A. V. β. *Vena pubo-ischio-trochanterici.*—This is a smaller vein, coming mainly from the pubo-ischio-trochanticus muscle and joining the lateral parietal vein just before it reaches the pelvic.

A. VI. *Venæ vesicæ* (text-figs. 12 and 13).—There appear to be generally five small vesicular veins, two entering each pelvic vein shortly before its union in the middle line, and the remaining one joining the base of the anterior abdominal.

B. *Vena iliaca communis* (GAUPP; V. ischiadica, HOCHSTETTER) (text-fig. 13).—Although in his second paper (46) HOCHSTETTER terms this vessel the vena ischiadica, there is no doubt it is the same vessel that in the Amphibia he calls vena iliaca (45). The latter name is preferable from the comparative point of view, since the same vessel receives this name in Elasmobranchs (59) and Amphibia (11 and 45), and one of its tributaries is the vena ischiadica of GAUPP (31), and this corresponds most nearly with the similarly named vessel in the Mammalia (*i.e.*, the former sciatic vein of the English anatomists). The common iliac vein is the main trunk draining the whole of the hind limb, after coming in from which it turns forwards and runs along for some distance parallel with the lateral wall of the kidney. It may be regarded as finishing near the front end of that organ, where it passes as a vena anastomotica (B. I), running in a cleft in the kidney into the afferent renal vein. Just at this point, as mentioned above, it gives rise to the large pelvic vein (*q.v.*), which looks like a continuation of the common iliac, but is better regarded as a tributary of it. At the hinder end of the kidney the common iliac sends a well marked tributary into the kidney substance (B. II). From this point it passes out towards the limb, receiving after a very short distance the ischiadic vein (B. III) from the deeper muscles of the external flexor side of the thigh. It now may be termed the *Vena iliaca externa* for a short way until it enters the limb as the *Vena femoralis*. It comes out on to the extensor surface of the thigh from between the ilio-femoralis and ischio-trochantericus muscles and, running mainly beneath the former, passes down to the knee, receiving three branches at its upper end (B. IV–VI). In the

lower part of this course it comes to lie close to the ischiadic artery, and may now be termed the *Vena poplitea*, and a number of branches (B. VII–X) enter it just above or just below the knee. It passes on down the extensor surface of the leg between the peroneus and extensor digitorum communis longus muscles, and may be called the *Vena peronea* down to the end of the body of the latter muscle, where it gives off a branch (B. XI), and runs on to the dorsal surface of the foot as the *Vena dorsalis pedis*. This forms an *Arcus venosus*, receiving a number of veins (B. XII–XV) from the extremity of the foot.

B. I. *Vena anastomotica renalis* (text-figs. 11 and 13).—As already noted, the front end of the iliac vein forms an anastomosing vessel that runs in a deep cleft between the first and second kidney lobes, thus putting the iliac and afferent renal vessels into direct communication. Just before turning mesiad, or just before entering the kidney substance, this vessel receives a factor (B. I. α) from the anterior end of the kidney. It is hard to say which way the blood flows in this anastomosing vein without an observation on a living specimen.

B. I. α. *Vena renalis anterior.*—This is a small factor, composed of several twigs from the first kidney lobe, and, from its constitution, looks as if it were efferent from the kidney.

B. II. *Vena renalis posterior* (text-fig. 13).—The posterior renal vein leaves the iliac and runs to the hinder kidney lobes, often giving off a fine vessel to that organ, before entering it. The direction of flow in this vessel also is not apparent, although it may be afferent to the kidney. It is not improbable that the direction of flow in both this and the preceding vein may vary in correlation with the relative changes of venous pressure in the kidney, limbs, and tail.

B. III. *Vena ischiadica* (? GAUPP).—A short distance before entering the limb, the main trunk receives a tributary, which may possibly correspond with the ischiadic vein in the frog, according to GAUPP, although, as he points out, it does not accompany the similarly named nerve. This vessel was not satisfactorily followed as in the examples examined; it was either not injected or did not contain sufficient blood to render it conspicuous. It comes from the deeper muscles on the external flexor side of the limb, and apparently drains the pubo-ischio-femoralis, pubo-ischio-trochantericus externus, pubo-ischio-tibialis and pubo-tibialis muscles, and also the deeper surfaces of the ischio-tibialis posticus and extensor triceps muscles.

The main trunk is now the *Vena iliaca externa* for a short way, and then enters the limb as the *Vena femoralis* (Plate 7, fig. 4; Plate 8, fig. 6).

B. IV. *Vena cutanea* 1 (Plate 8, fig. 6).—The first cutaneous vein is a small superficial factor, joining the main trunk high up between the ileo-femoralis and ischio-trochantericus muscles.

B. V. *Ramus muscularis* 1.—This is a larger vein, joining the femoral a little below the preceding, and composed of two main factors: one from the extensor side of the ischio-tibialis muscle and one from the ischio-tibialis-posticus.

B. VI. *Ramus muscularis* 2.—The second muscular vein is about the same size as the first, and enters about half way down the thigh. It is also composed of two branches, one from the ilio-femoralis and one from the external, lower portion of the extensor triceps muscle.

Some distance below this the main trunk is called the *Vena poplitea* (Plate 7, fig. 4; Plate 8, fig. 6).

B. VII. *Ramus muscularis* 3.—This is a short muscular trunk coming from the lower posterior part of the extensor triceps muscle.

B. VIII. *Vena cutanea* 2.—The second cutaneous vein comes from the superficial tissues close to the knee, and enters the main trunk just above the joint.

B. IX. *Ramus muscularis* 4.—A fairly large muscular branch comes from between the gastrocnemius and peroneous muscles, which it drains, and joins the popliteal vein almost at the knee joint.

B. X. *Vena circumflexa genu lateralis inferior* (GAUPP) (Plate 8, fig. 6).—The circumflex vein corresponds closely with the similarly named vessel in the frog. It enters the popliteal by two short vessels, and drains the tendinous capsule of the knee and the insertion of the extensor triceps muscle.

The main trunk is now termed the *Vena peronea* (Plate 7, fig. 4; Plate 8, fig. 6).

B. XI. *Vena anastomotica arcus venosi* (Plate 8, fig. 6).—The anastomosing vein leaves the end of the peroneal, and runs downwards and mesiad, to enter the mid region of the arcus venosus, just opposite to the interdigital vein of digits three and four.

The main trunk now becomes the *Vena dorsalis pedis* (Plate 8, fig. 6).

B. XII. *Vena circumflexa tarsi* (GAUPP) (Plate 8, fig. 6).—The tarsal circumflex vein passes round over the distal extremity of the peroneus muscle on to the plantar surface of the foot, where it is probably connected with the plantar veins, although these could not be satisfactorily made out. It receives a small twig from the outer side of the fifth digit.

B. XIII. *Venæ interdigitales* (Plate 8, fig. 6).—Three well marked interdigital veins enter the arcus venosus. The first and second come from the spaces between the fourth and fifth and third and fourth digits respectively. The

third is a very short trunk, formed by the union of the two veins related to the space between digits two and three and one and two.

B. XIV. *Vena præ-pollicis.*—This vein runs up the external border of the big toe.

B. XV. *Ramus muscularis* 5.—The muscular vein comes from between the distal ends of the tibialis anticus and extensor digitorum communis longus muscle. It joins with the foregoing to form the distal end of the arcus venosus.

The System of the Epigastric Veins (text-fig. 12).—Unfortunately it was not possible to make out the details of the epigastric veins, which are never easy to dissect. They were not successfully injected in any of the specimens, and they are hidden by the dense black pigmentation of the peritoneum. Certain parts of the system, however, were traced fairly satisfactorily. The epigastric veins in the Lacertilia differ very considerably from species to species, according to the observations of BEDDARD. The most common arrangement is apparently a median epigastric vein running in the mid-ventral line, such a vessel is found, for example, in *Tiliqua scincoides* (2), *Phelsuma madagascariensis* (4), *Heloderma suspectum* (8), and *Ophisaurus apus* (7). When paired or lateral epigastric veins are present their anterior and posterior connections differ so considerably that there is apparently no common arrangement. Paired epigastric veins are present in *Varanus griseus* (8), and in yet other forms, *Iguana tuberculata* (2), *Tupinambis nigropunctatus* (3), and *Chamæleon vulgaris* (4), both lateral and median epigastric veins occur.

Sphenodon possesses a well marked median and smaller lateral epigastric veins.

Vena epigastrica lateralis (text-fig. 12).—The lateral epigastric vein is a small vessel running in the ventral abdominal wall in the region of the epigastric artery. Just behind the liver it unites with its fellow in the middle line to form the median epigastric vein. The anterior and posterior connections of this vessel could not be ascertained. In the short part of its course, over which it was traced, it was found to receive several small parietal factors. In the case of *C. vulgaris* there is a somewhat similar relation between lateral and median epigastrics, for the two former unite to form the latter, only in this case it is in front of the liver.

Vena epigastrica media (text-fig. 12).—The median epigastric vein is a somewhat short but well marked vessel formed, as far as could be seen, by the union of the two factors from the lateral epigastrics, and running forward to the hinder end of the liver. Here the main part of it enters the anterior abdominal vein, just before this joins the hepatic portal, while a smaller part passes on forward in the mid-ventral line, but it could only be followed for a short distance. In *Ophisaurus apus* the median epigastric is in two portions, and the posterior part is related to the anterior abdominal vein in a manner similar to that in *Sphenodon.* When a median epigastric vein is present it is generally related to the anterior abdominal vein.

Venæ Pulmonales (text-fig. 1).

The pulmonary veins are two moderate sized thin-walled vessels running up the ventro-lateral borders of the lungs, one on each side of the body. They increase in size as they receive numerous tributaries from the substance of the lungs themselves. Leaving the anterior end of the lungs they pass dorsally to the sinus venosus, to become closely attached to its anterior border on the atrial walls. As has been pointed out previously, they open into the postero-mesial corner of the left atrium by an aperture partly hidden by a fold of the atrial wall and quite close to the inter-atrial septum.

Summary.

The heart is on the whole reptilian but of a simple unspecialised type, in which the three main arterial vessels instead of opening directly from the ventricle come off by a short common trunk, possibly a remains of the conus arteriosus.

The arterial system in general is distinctly reptilian, and while in some respects it may recall conditions in other orders of Reptilia, it most closely resembles that of certain Lacertilians, but it is undoubtedly less specialised and shows certain interesting points of similarity with that of the Urodeles.

The same general remarks also apply to the veins, which are more primitive than those of Lacertilia, although they approximate more nearly to the latter than to other Reptiles.

Certain special points in the blood vessels will be summarised below when considering the light they throw upon the position of *Sphenodon* in the class Reptilia. It only remains to note that the blood vascular system of the Tuatara is of considerable anatomical interest, since looked at broadly it is much more primitive than that of any other Reptile so far described. On the one hand, it has resemblances to the Crocodilia, Chelonia, particularly the Lacertilia, and even the Ophidia, while on the other hand it recalls in a number of striking features that characteristic of the Urodela. In it then we have an arrangement that should be borne in mind when dealing with other forms, and one that is essential to a proper appreciation of the disposition of the vessels in the Lacertilia.

Conclusions.

As was pointed out in the introduction, two views are commonly held with regard to the systematic position of *Sphenodon*. The more common and perhaps more orthodox view, since it is the one put forward in most text-books, is that *Sphenodon* is the sole modern representative of an order of the Reptilia termed the Rhynchocephalia, equal in rank to the other four orders of the class. This suggestion was first put forward by Günther (41) in 1867. As far as palæontological records show, the order is ancient but was never an extensive one. On this ground there is no inherent improbability in its only remnant being found in Australasia, since this region has

furnished other examples of orders of animals either poorly represented or not represented at all in other parts of the world. The second view put forward by HUXLEY, among others, is that the differences between *Sphenodon* and some of the least specialised Lacertilia are not so great as to justify placing it in a separate order, but, on the contrary, it should be included in the Lacertilia. It is not intended in the present paper to discuss fully all the evidence for and against these two views, but at the same time, in the course of comparing the blood vessels with those of a number of other Reptiles, it was inevitable that some attention had to be paid to evidence put forward by various writers as to the systematic position of the animal.

A full enumeration of the characteristic features of *Sphenodon* and discussions as to their value will be found in the works of BOULENGER (16), BUSCH, GADOW and HOWES and SWINNERTON, and they need not be repeated here. One or two points have been raised subsequently and call for brief notice.

GADOW (29) pointed out that *Sphenodon* lacks distinct copulatory organs, and so is to be regarded as a primitive type, and he even pointed out similarities between the cloaca in this form and in the Gymniophiona. Later, OSAWA (63) sought to homologise the cloacal scent glands with the copulatory organs of other Reptiles, and concludes (p. 346), ". . . muss ich mit der Behauptung auftreten, dass der *Hatteria* auch ein Begattungsorgan zukommt." The impossibility of homologising the scent glands with copulatory organs, for both may occur together in Snakes, was subsequently reaffirmed by GADOW, who concluded (30, p. 43): "*Hatteria* ist und bleibt das niederste lebende Reptil welches wir kennen; es gehört weder zu den Krokodilen, was niemand behaupten wird, noch zu den Saurien, . . ."

Another of these features is the characteristic forward extension of the pterygoid bones to meet the vomers, so that the palatines are, as it were, pushed to the side and excluded from participation in the formation of the middle portion of the bony palate. This, as HOWES and SWINNERTON point out, is "a feature already recognisable among the Batrachia and Stegocephalia." BEDDARD (6) afterwards described the skull of *Uromastix spinipes*, in which the pterygoids pass forwards almost, if not actually, to touch the vomers, and so called attention to the fact that this character, supposed to be peculiar to "Hatteria," is also found in a Lacertilian. This condition is very unusual in the Lacertilia; indeed, it does not appear to be found in any other species that has been described, not even in the closely allied *Uromastix hardwickii*, according to BUSCH (20). It cannot therefore be urged as showing any relationship between *Sphenodon* and the Lacertilia, for it is distinctly atypical of that order, but is better regarded as (so far as known) an isolated example of a species of Lizard that has retained a Batrachian condition that is also found in *Sphenodon* in a more complete form.

We are here more concerned with the blood vascular system than with other anatomical features, and it will simplify matters if we glance briefly at the points that have come to light in comparing *Sphenodon* with other forms. In other

Reptilia the three main arterial trunks come off separately from the ventricle, while in the Tuatara they come off from a short common trunk, probably representing the conus arteriosus of the Amphibia which has been lost in other Reptilia.

Sphenodon possesses both a ductus caroticus and a ductus arteriosus (BOTALLI) on each side, and they are in a moderately developed condition. That this is a primitive condition is shown by the fact that it occurs in the Amphibia Urodela, and is not found, so far as is known, in any other reptile. The carotids, in origin and general distribution, and also in the absence of a common trunk, *i.e.*, a carotis primaria, resemble those of the Urodele Amphibia, and also some of the primitive Lacertilia. The same is also true of the arteria laryngeo-trachealis, which comes off from the pulmonary artery in Amphibia and in certain Lizards. This vessel appears to be homologous with the arteria œsophagea of the Urodeles, and so is a relic of the time when respiration took place in the mucous membrane of the buccal cavity and pharynx. VAN BEMMELEN (10, p. 102) gives his opinion of the neck region as follows: "Vor allem stellte es sich heraus, dass *Hatteria*, im Baue ihrer Halsgegend eine typische Eidechse ist, und darin besonders mit den Ascalaboten sehr übereinstimmt." As was noted previously, he was mainly concerned with the nerves. DENDY (22) gives a comparison between the intra-cranial arteries of *Sphenodon* and other reptiles. He notes several points of difference from the Lacertilia, but, nevertheless, records the general impression (p. 411) that "the arrangement of the cerebral arteries in *Sphenodon* strongly supports the view that that animal is closely related to the Lacertilia, though it approaches the more primitive condition of the Chelonia as regards the basilar artery."

In regard to the gut arteries, HOCHSTETTER, in his comparative account of these vessels in Saurians, says (44, p. 217): "Dabei werde ich von *Hatteria* ausgehen, weil bei dieser Form entschieden die ursprünglichsten Verhältnisse bei *Lacerta* von denen bei *Hatteria* in einfacher Weise ableiten lassen." The same conclusion is put forward by GEGENBAUR (33, footnote, p. 536).

The arteria interossea leaves the extensor surface of the fore foot and passes through between the ends of the radius and ulna on to the flexor surface. It returns again, passing through the wrist between the carpal bones. This rather striking course is also primitive, since it is met with in the Amphibia.

Certain points in the relationships of the veins also deserve consideration. The paired venæ tracheales are in a more primitive condition than in *Lacerta*, and, indeed, represent a stage that is actually passed through by *Lacerta* in the course of its development. In all probability they are homologous with the venæ pharyngeæ in Urodele Amphibia. The vena cerebralis posterior in *Sphenodon* leaves the cranium by the foramen jugulare in company with the tenth cranial nerve, a very important point, as this vein is more or less constant in position throughout the vertebrate series. It accompanies the vagus nerve in Elasmobranchs, Urodele and Anurous Amphibia, Birds and Mammals, so that it must be regarded as primitive and

fairly constant. In Lacertilia, and, as far as is known, Ophidia, this vessel leaves the cranium through the foramen magnum, and is thus separated from the vagus. This, then, is a significant difference between *Sphenodon* and the Lacertilia. The vena cerebralis media and the vena capitis dorsalis are both to be regarded as primitive, since they characterise the primitive Amphibia and Reptilia. DENDY (22) has noted several points which, in my opinion, constitute considerable differences between the intra-cranial veins of *Sphenodon* and the Lacertilia, and points out (p. 423) that "a very characteristic feature of *Sphenodon* is the development of large transverse sinuses resembling those of the Crocodile, but these communicate with the extra-cranial vascular system in quite a different manner from that described by RATHKE in the latter animal." There is present a sinus orbito-nasalis, corresponding with a vein of the same name in the Amphibia and Elasmobranchs, and this is not found in *Lacerta* or *Tropidonotus*.

In *Sphenodon* the state of development of the vena azygos and the supra-renal portal system, and also the relations of these vessels, which are remnants of the embryonic posterior cardinal vein, are more primitive than in *Lacerta*, and indeed present a condition from which any of the arrangements met with in the Lacertilia can be derived. The anterior gastric vein resembles that of the Urodele Amphibia, and although present in an Ophidian *Eryx*, does not appear to have been described in Lacertilians. The constitution of the anterior abdominal vein, while resembling that in *Lacerta* to a certain extent, and also the Urodela, is more primitive than in any member of the order to which the former belongs. BEDDARD, as the result of his work on the body veins of *Sphenodon*, concludes (7, p. 462) :—"There is no question that, apart from details, the venous system of *Hatteria* is distinctly Lacertilian. Nor do the differences which it shows from the Lacertilia tend to prove a nearer resemblance to the Chelonia or to the Crocodilia. On the other hand, I believe it possible to detect likenesses to the Ophidia. This, however, in my opinion, does not argue a special affinity between *Hatteria* and the Ophidia, but the antiquity of the *Hatteria* type which Palæontology, as is well known, has proved." Notwithstanding this view of one who has a very extensive knowledge of the blood vessels in the Reptilia, it seems to me that, when a more extended survey is taken and the many points of similarity between the vessels of *Sphenodon* and the Amphibia, especially the Urodela, are borne in mind, the above conclusions hardly go far enough, and that the primitiveness of *Sphenodon* is considerably more pronounced than they appear to indicate.

Thus, I think, it will be seen that in a large number of points, some of considerable importance, the vascular system of the Tuatara is more primitive than that in any of the Lacertilia. Indeed, if we consider the cumulative effect of all these points, it seems inevitable that we must regard the vascular system as a whole as being of a much less specialised type, so much so that it merits the placing of the animal in a separate order. Certain features need re-emphasising, the unspecialised condition of

the heart, the retention of the ductus caroticus and arteriosus, and the position of the posterior cerebral vein. In this last matter, *Sphenodon* resembles both the higher and lower Vertebrates, while the Lacertilia have departed from this condition. Also, there does not appear to be any primitive condition in the vessels of any Lacertilian that is not also found in *Sphenodon;* and yet, again, in its intra-cranial vessels it gives a suggestion of the conditions realised in the Chelonia and the Crocodilia, which is just what might be expected of a primitive form.

The fact that *Sphenodon* resembles the Lacertilia in certain respects, not alone in the blood vessels, as BEDDARD has pointed out, does not, I submit, justify its inclusion in this order any more than its likenesses to the Urodela, which are many, demand its being placed with them. Indeed, the fact that *Sphenodon* has structures to be found in various Lacertilians, but not all in the same species, or even in the same genus, seems to suggest, and rather forcibly too, that when such characters are encountered in one of the Lacertilia it indicates that, in this particular respect, the Lizard has retained a primitive condition which is present in the Tuatara, and that the latter animal is more primitive than any of them. The Lacertilia differ among themselves in the disposition of their blood vessels, but when they do it is the result of modification or specialisation; whereas practically always when *Sphenodon* differs from them it will be found to be in a way that either suggests conditions in other Reptiles or most frequently approximates it more nearly to the Urodela.

The object of the present investigation was in the first place to give a fairly complete account of the blood vascular system of the Tuatara, as, owing to its rarity, an opportunity of examining a well preserved series of specimens might not occur again. Secondly, no such complete account is available for any Lacertilian, and as a large amount of comparative work has been done on various points in different species of Reptiles, it seemed desirable to correlate this with such an admittedly little specialised type to form a basis for future comparative work.

At the outset I was inclined to regard *Sphenodon* as in all probability a lowly member of the order Lacertilia. The condition of its vascular system, briefly epitomised above, however, was found to be so primitive in all respects, and to differ so much from that in the Lacertilia in certain important features, that it was obvious that it could not be included in that order. Reference to the somewhat extensive literature regarding other points in its anatomy disclosed no valid reason that would necessitate its being placed among the Lacertilia; but, on the other hand, it brought out a number of points (more than need be dealt with here) to show it to be a more primitive type of Reptile. I have therefore been led to conclude that while *Sphenodon* approaches the Lacertilia more closely than any other order of the Reptilia, it nevertheless differs from them to such an extent that GÜNTHER was thoroughly justified in placing it in a separate order, the Rhynchocephalia, and that it is the most primitive living Reptile.

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DESCRIPTION OF PLATES.

Plate 6.

Fig. 1.—Diagrammatic sketch of a general view of the viscera of Sphenodon, with the alimentary canal displaced to the right.

Vessels.

A.C., arteria cœliaca; *A.E.A.*, arteria epigastrica anterior; *A.I.C.*, arteria iliaca communis; *A.Il.E.*, arteria iliaca externa; *A.L.*, arteria lumbalis; *A.M.C.*, arteria mesenterica communis; *A.S.*, arteria scapularis; *C.E.*, carotis externa; *C.I.*, carotis interna; *D.Ao.*, dorsal aorta; *R.E.A.* 1, ramus muscularis of arteria epigastrica; *V.*, ventricle; *V.A.A.*, vena abdominalis anterior; *V.Az.*, vena azygos; *V.C.A.S.*, vena cava anterior sinistra; *V.Co.C.*, vena coraco-clavicularis; *V.C.P.*, vena cava posterior; *V.Cu.L.*, vena cutanea lateralis; *V.Cu.M.*, Vena cutanea magna; *V.Il.*, vena iliaca communis; *V.J.E.*, vena jugularis externa; *V.J.I.*, vena jugularis interna; *V.Pl.*, vena parietalis lateralis; *V.R.R.*, venæ renales revehentes; *V.Sc.*, vena subclavia; *V.Sp.D.*, vena spermatica dextra; *V.Sp.S.*, vena spermatica sinistra; *V.Ve.*, venæ vesicæ.

Other Structures.

Blad., bladder; *G.Blad.*, gall bladder; *Int.*, intestine; *Liv.*, liver; *Mesor.*, mesorchium; *Rect.*, rectum; *Rib.*, cut end of ribs; *Rt.Kid.*, right kidney; *Rt.Lung*, right lung; *Rt.Tes.*, right testis.

PLATE 7.

Fig. 2.—Diagrammatic sketch of the vessels shown in a lateral dissection of the head, certain of the bones having been removed.

Vessels.

A.A., arteria articularis; *A.Au.*, arteria auricularis; *A.F.*, arteria frontalis; *A.Mn.*, arteria mandibularis; *A.M.G.*, arteria musculo-glandaris; *A.Mn.E.*, arteria mandibularis externa; *A.Mn.I.*, arteria mandibularis interna; *A.N.*, arteria nasalis; *A.Oc.*, arteria occipitalis; *A.O.I.*, arteria orbitalis inferior; *A.Op.*, arteria ophthalmica; *A.O.S.*, arteria orbitalis superior; *A.T.*, arteria temporalis; *R.C.*, ramus coronoideus; *R.C.I.*, 1 and 2, rami musculares of arteria temporalis; *R.Mn.* 1, 2 and 3, rami musculares of arteria mandibularis; *R.m.n.*, ramus membranæ nictitantis; *R.t.m.*, ramus temporo-masseteris; *S.M.N.*, sinus membranæ nictitans; *S.O.*, sinus orbitalis; *V.Ca.L.*, vena capitis lateralis; *V.Ce.M.*, vena cerebralis media; *V.Ce.P.*, vena cerebralis posterior; *V.Ce.R.*, ramus muscularis of vena cerebralis posterior; *V.J.I.*, vena jugularis interna; *V.J.R.* 2–6, rami musculares of vena jugularis interna; *V.Mn.*, vena mandibularis; *V.Mn.I.*, vena mandibularis interna; *V.Mn.R.*, rami musculares of vena mandibularis; *V.Oc.*, vena occipitalis; *V.Pt.*, vena pterygoidea; *V.S.*, vena supra-temporalis; *V.T.A.*, vena tympanica anterior; *V.T.A.R.*, ramus muscularis of vena tympanica anterior.

Other Structures.

1, cleido-humeralis muscle; 2, ventral part of levator scapulæ; 3, dorsal part of levator scapulæ; 4, longissimus; 5, cucullaris; 6, cut end of parietal bone; 7, parietal bone; 8, columella cranii; 9, cut end of post-frontal bone; 10, rectus superior; 11, frontal bone; 12, obliquus superior; 13, Harderian gland; 14, obliquus inferior; 15, rectus inferior; 16, cut end of jugal bone; 17, coronoid process of mandible; 18, cut end of quadrato-jugal bone; 19, cut end of squamosal and quadrate bones; 20, anterior hyoid bone; 21, posterior hyoid bone; *V*, nervus trigeminus; *X*, vagus.

Fig. 3.—Diagrammatic sketch of the arteries and veins shown in a dissection of the head and neck from the ventral side.

Vessels.

A.Gg., arteria genio-glossa; *A.Gp.*, arteria glossopharyngeus; *A.L.T.*, arteria laryngeo-trachealis; *A.M.*, arteria muscularis cervicis; *A.Oe.*, arteria œsophagea; *A.P.S.*, arteria pterygoideus superficialis; *A.S.M.*, arteria submandibularis; *A.T.I.*, arteria thyreoidea inferior; *A.T.S.*, arteria thyreoidea superior; *C.C.*, carotis communis; *C.E.*, carotis externa; *C.I.*, carotis interna; *D.C.*, ductus caroticus; *L.S.A.*, left systemic arch; *R.m.m.*, ramus musculo-mandibularis; *S.A.*, systemic arch; *V.C.A.D.*, vena cava anterior dextra; *V.J.C.D.*, vena jugularis communis dextra; *V.J.C.S.*, vena jugularis communis sinistra; *V.J.E.*, vena jugularis externa; *V.J.I.*, vena jugularis interna; *V.J.R.* 1–5, rami musculares of vena jugulares; *V.O.*, venæ œsophageæ; *V.Sc.*, vena subclavia; *V.T.R.I.*, ramus muscularis of vena trachealis.

Other Structures.

Cer.Sym., cervical sympathetic; *Gen.gl.*, genio-glossus; *Gen.hy.*, genio-hyoid; *Hy.* 1, anterior cornu of hyoid; *Hy.* 2, posterior cornu of hyoid; *Hyo-man.*, hyo-mandibularis; *Int.Pte.*, internal pterygoid; *Lev.Hy.*, levator hyoidens; *Plat.*, platysma; *Rec.Lar.X.*, recurrent laryngeal of vagus; *Tra.*, trachea; X., vagus; XII., hypoglossal.

PLATE 8.

Figs. 4–10, Diagrammatic Sketches—

Fig. 4.—Vessels of the left hind limb viewed from the extensor surface.

Fig. 5.—Deeper arteries of the right hind foot viewed from the flexor (plantar) side.

Fig. 6.—Veins of the right hind limb viewed from the extensor surface.

Fig. 7.—Veins of the right fore limb viewed from the extensor surface.

Fig. 8.—Vessels of the left fore limb viewed from the extensor surface.

Fig. 9.—Vessels of the left fore limb viewed from the flexor surface.

Fig. 10.—Relation of the arteries to the muscles of the eye viewed from the inner aspect.

Muscles of Hind Limb.

5, ileo-femoralis; 9, ischio-trochantericus; 11, ischio-tibialis posticus; 13, ilio-fibularis; 14, extensor triceps; 15, gastrocnemius; 16, flexor digitorum communis profundus; 17 and 20, tibialis anticus; 21, extensor digitorum communis longus; 22, peroneus; 23, abductor et extensor hallucis longus; 29, abductor digitorum communis; 32, interossei planteres.

Arteries of Hind Limb.

A.C.F.E., arteria circumflexa femoris externa; *A.C.F.I.*, arteria circumflexa femoris interna; *A.C.G.E.*, arteria circumflexa genu externa; *A.C.G.I.*, arteria circumflexa genu interna; *A.D.P.*, arteria dorsalis pedis; *A.I.*, arteriæ interdigitales; *A.I.E.*, arteria interossea externa; *A.Io.*, arteria interossea; *A.Is.*, arteria ischiadica; *A.M.E.*, arteria metatarsalis externa; *A.M.I.*, arteria metatarsalis interna; *A.P.P.*, arteria perforans plantaris; *A.T.L.*, arteria tibialis lateralis; *A.T.M.*, arteria tibialis media; *R.I.C.* 1 and 2, rami musculares 1 and 2 of the arteria iliaca communis.

Veins of Hind Limb.

V.A.V., vena anastomotica arcus venosi; *V.C.G.*, vena circumflexa genu lateralis inferior; *V.C.T.*, vena circumflexa tarsi; *V.Cu.* 1 and 2, venæ cutaneæ; *V.D.P.*, vena dorsalis pedis; *V.D.R.* 5, ramus muscularis of the vena dorsalis pedis; *V.F.*, vena femoralis: *V.F.R.* 1 and 2, rami musculares of the vena femoralis; *V.I.*, venæ interdigitales; *V.Pe.*, vena peronea; *V.Po.*, vena poplitea; *V.Po.R.* 4, ramus muscularis of the vena poplitea; *V.P.P.*, vena præ-pollicis.

I Pollex; II–V digits (figs. 4–6).

Muscles of Fore Limb.

2, latissimus dorsi; 11, pectoralis; 12, cleido-humeralis; 13, dorsalis scapulæ; 14, supra-coracoideus; 15, coraco-brachialis; 17, scapulo-humeralis posterior; 18, scapulo-coraco-brachialis; 19, coraco-antibrachialis; 22, anconæus; 23, flexor-carpi-radialis; 24, flexor-digitorum-communis-profundus; 26, pronator teres; 27, pronator quadratus; 28, supinator; 29, extensor carpi radialis brevis; 30, extensor digitorum communis longus; 31, extensor carpi ulnaris; 32, anconæus quartus (richtiger quintus); 33, extensor carpi radialis longus; 34, abductor et extensor pollicis longus; 35, flexor digitorum communis sublimis; 43, extensor digitorum communis brevis.

Arteries of Fore Limb.

A.B., arteria bicipitalis; *A.I.*, arteriæ interdigitales; *A.I.E.*, arteria interossia externa; *A.Io.*, arteria interossea; *A.U.*, arteria ulnaris; *A.V.*, arteria volaris; *A.V.P.*, arcus volaris profundus; *R.r.*, ramus radialis; *Rr.* 1, ramus radialis of arcus dorsalis; *Ru.*, ramus ulnaris; *Ru.* 1, ramus ulnaris of arcus dorsalis.

Veins of Fore Limb.

A.V.D., arcus venosus dorsi manus; *V.A.L.*, vena anastomatica longa; *V.A.S.*, vena antibrachialis superficialis; *V.A.V.*, vena anastomatica arcus venosi; *V.B.*, vena branchialis; *V.B.R.*, *V.B.R.* 2, rami musculares of vena brachialis; *V.Cu.M.*, vena cutanea magna; *V.I.*, vena interdigitales; *V.Io.*, vena interossea; *V.P.B.*, vena profunda brachii; *V.P.H.*, vena præ-hallucis.

Sketch of Dissection of Eye from inner aspect (fig. 10).

Arteries.

A.F., arteria frontalis; *A.M.G.*, arteria musculo-glandaris; *A.N.*, arteria nasalis; *A.Op.*, arteria ophthalmica; *A.O.S.*, Arteria orbitalis superior; *C.* 1, branch to posterior rectus; *C.* 3, branch to superior rectus; *C.* 4, branch to back of eye; *C.* 5, branch to anterior rectus; *C.* 6, branch to inferior rectus.

Muscles.

Rect.Ant., rectus anterior; *Rect.Inf.*, rectus inferior; *Rect.Post.*, rectus posterior; *Rect.Sup.*, rectus superior; *Ret.Bul.*, retractor bulbi; *Obl.Inf.*, obliquus inferior; *Obl.Sup.*, obliquus superior; *Hard.Gl.*, Harderian gland; *II.*, optic nerve.

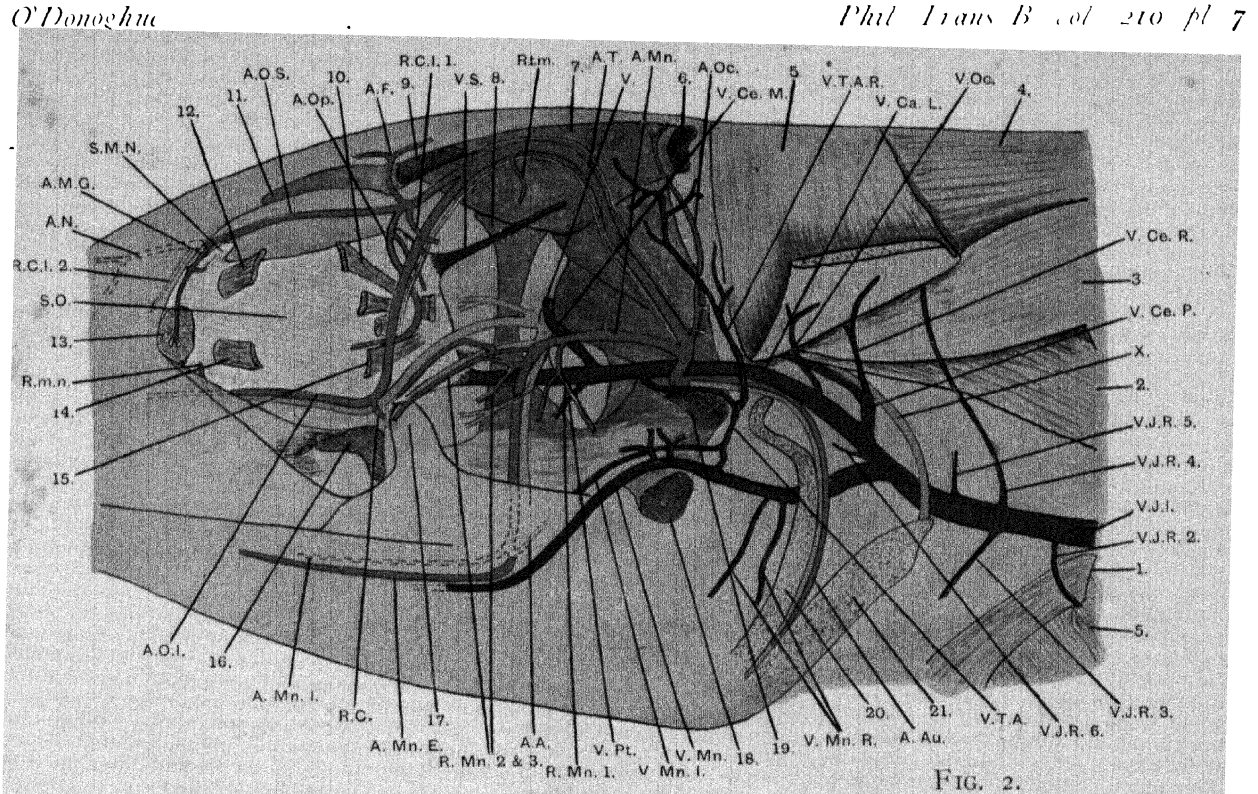

A.O.S.
10.
R.C.I. 1.
Rtm.
7.
A.T.
A.Mn.
A.Oc.
5.
V.T.A.R.
V.Oc.
4.
11.
A.Op.
A.F.
9.
V.S.
8.
V.
6.
V. Ce. M.
V. Ca. L.
12.
S.M.N.
A.M.G.
A.N.
R.C.I. 2.
S.O.
13.
R.m.n.
14.
15.
V. Ce. R.
3.
V. Ce. P.
X.
2.
V.J.R. 5.
V.J.R. 4.
V.J.I.
V.J.R. 2.
1.
5.
A.O.I.
16.
A. Mn. I.
R.C.
17.
A. Mn. E.
R. Mn. 2 & 3.
A.A.
R. Mn. 1.
V. Pt.
V. Mn.
V. Mn. I.
18.
19.
V. Mn. R.
20.
A. Au.
21.
V.T.A.
V.J.R. 6.
V.J.R. 3.

FIG. 2.

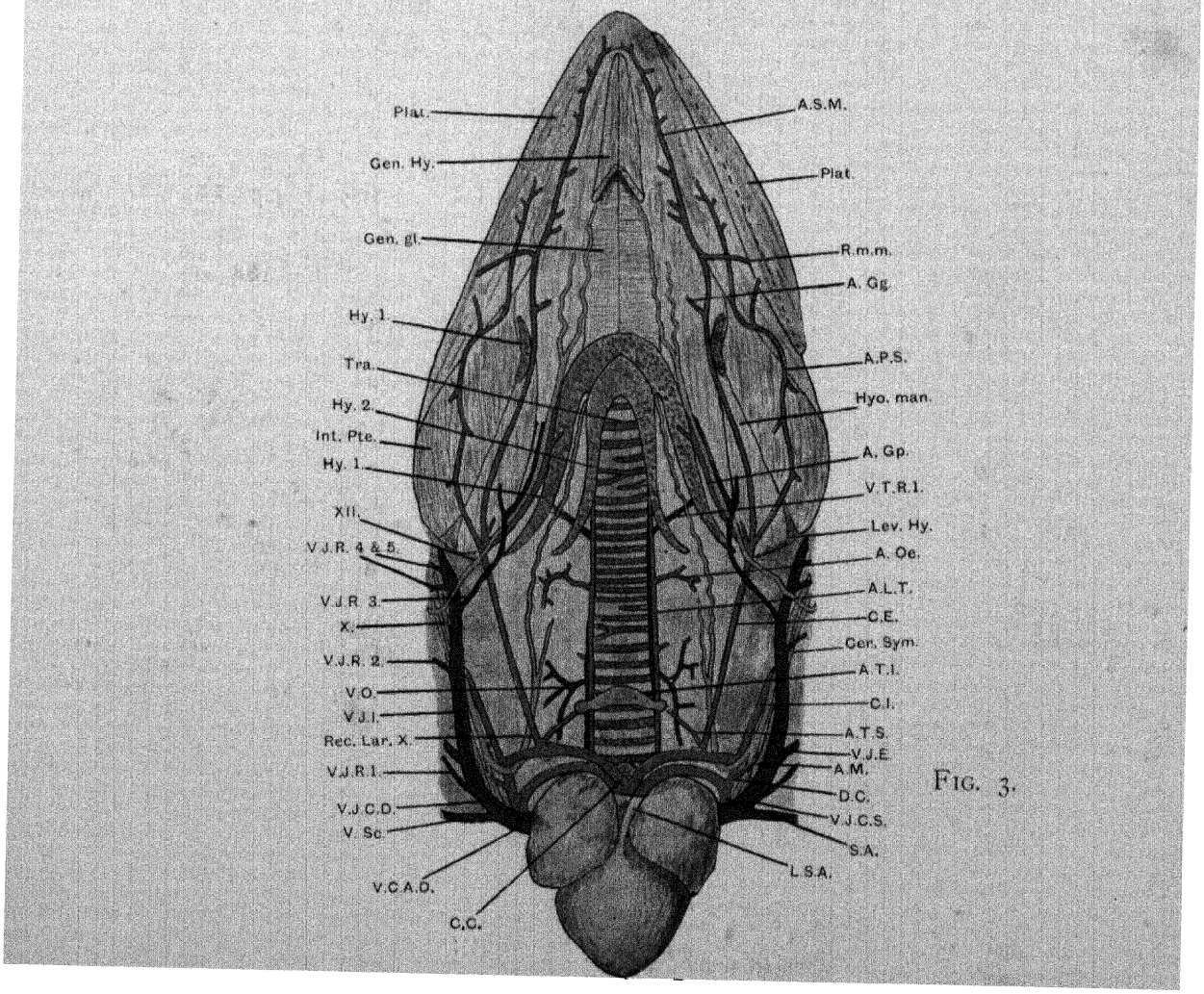

Plat.
A.S.M.
Gen. Hy.
Plat.
Gen. gl.
R.m.m.
A. Gg.
Hy. 1.
Tra.
A.P.S.
Hyo. man.
Hy. 2.
Int. Pte.
A. Gp.
Hy. 1.
V.T.R.1.
XII.
Lev. Hy.
V.J.R. 4 & 5.
A. Oe.
A.L.T.
V.J.R. 3.
C.E.
X.
Cer. Sym.
V.J.R. 2.
A.T.I.
V.O.
C.I.
V.J.I.
A.T.S.
Rec. Lar. X.
V.J.E.
V.J.R.1.
A.M.
D.C.
V.J.C.D.
V.J.C.S.
V. Sc.
S.A.
L.S.A.
V.C.A.D.
C.C.

FIG. 3.

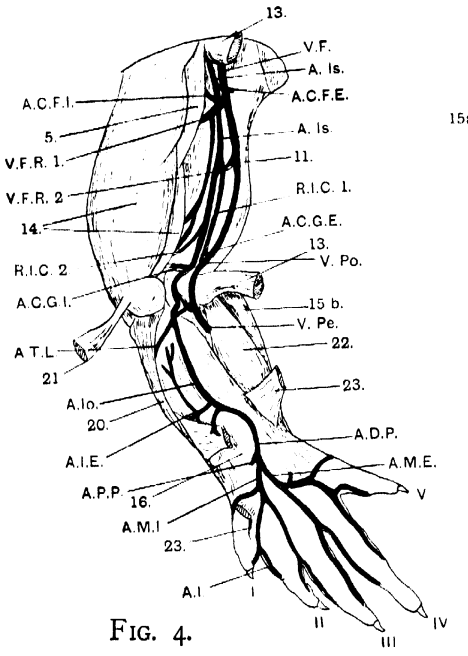


FIG. 4.

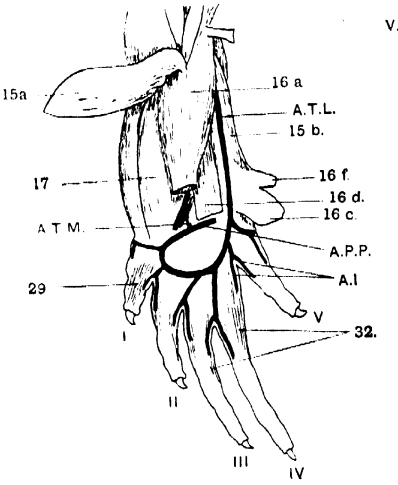


FIG. 5.

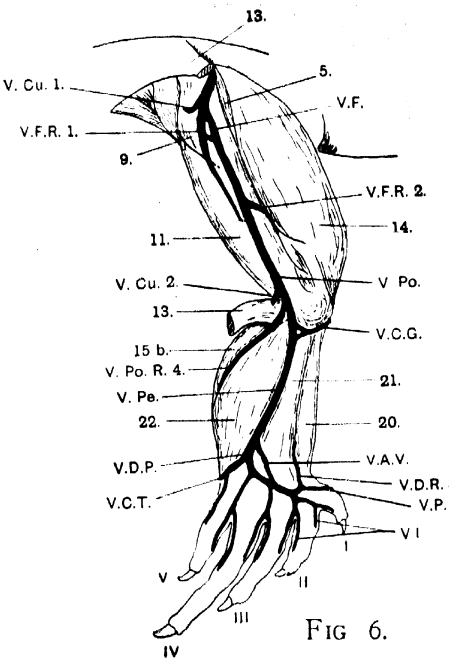


FIG 6.

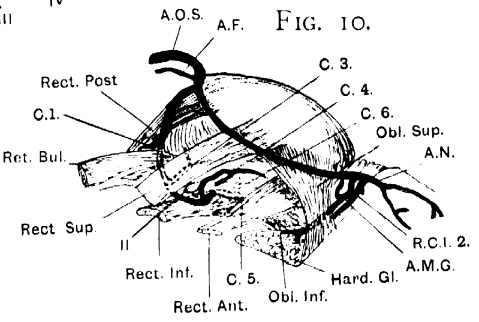


FIG. 10.

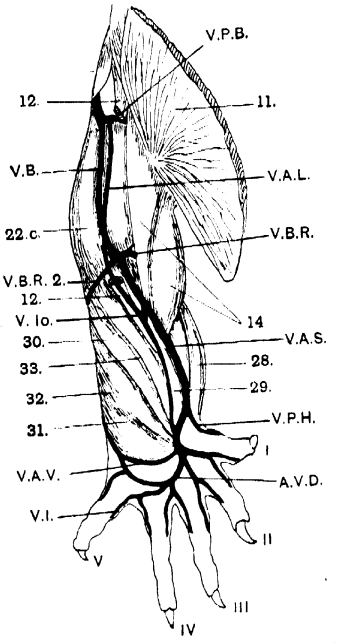


FIG. 7.

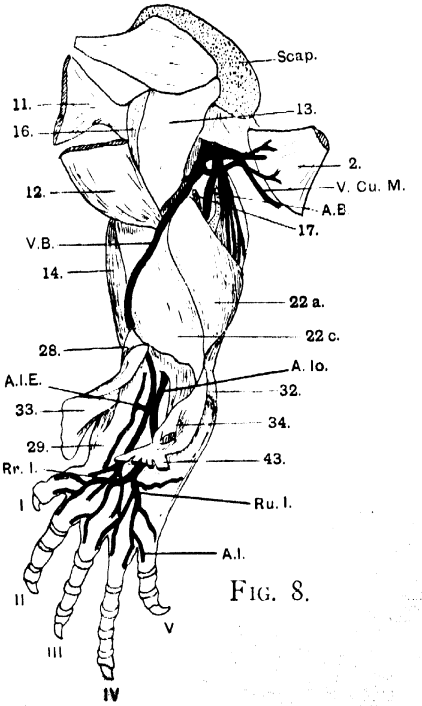


FIG. 8.

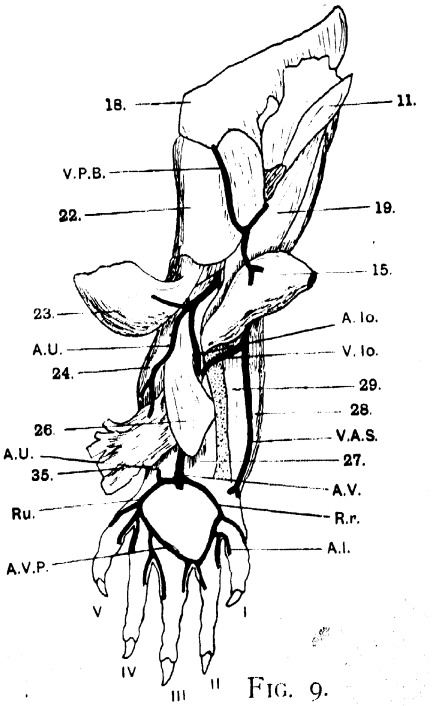


FIG. 9.

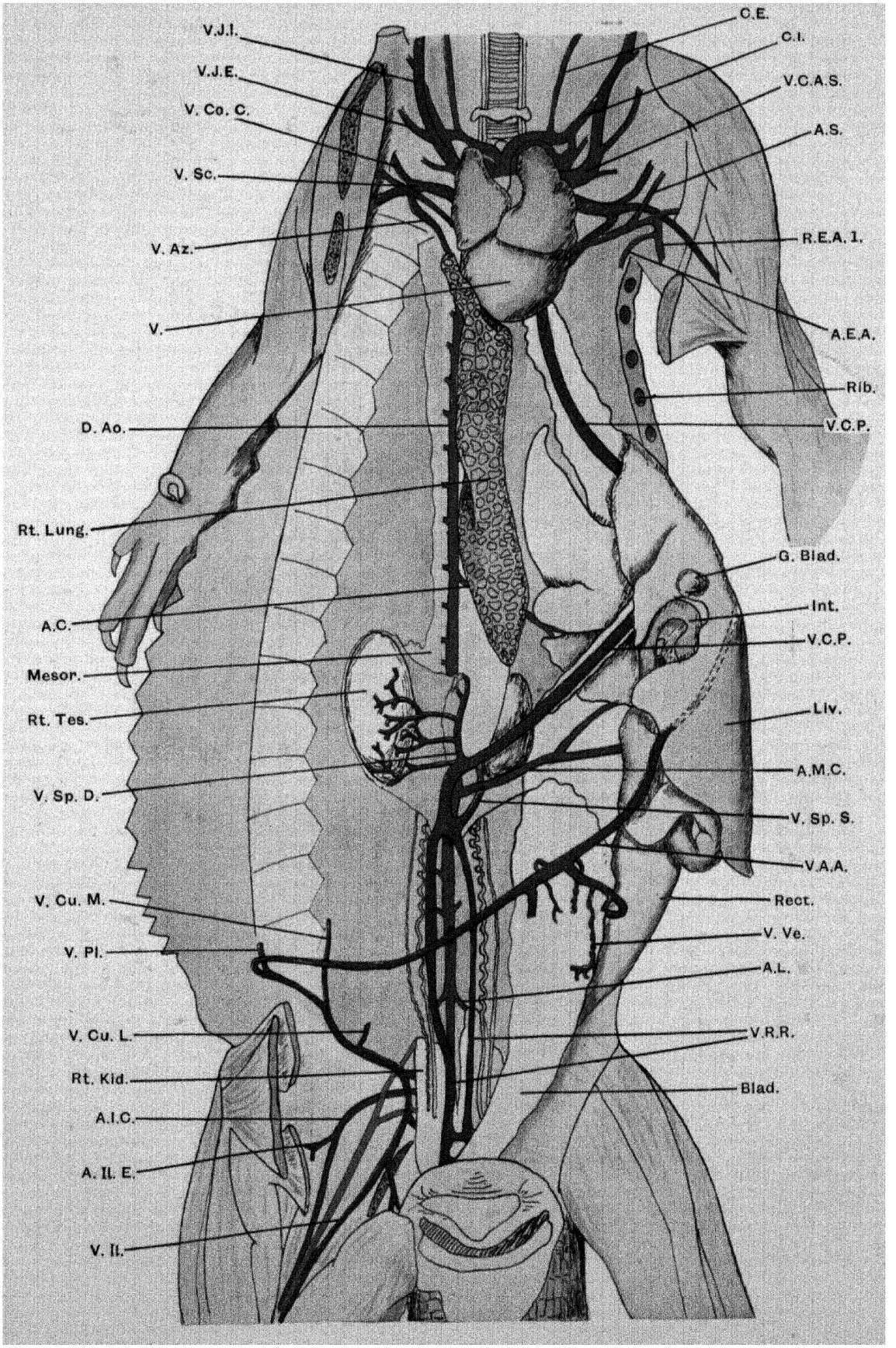

V.J.I.
V.J.E.
V. Co. C.
V. Sc.
V. Az.
V.
D. Ao.
Rt. Lung.
A.C.
Mesor.
Rt. Tes.
V. Sp. D.
V. Cu. M.
V. Pl.
V. Cu. L.
Rt. Kid.
A.I.C.
A. Il. E.
V. Il.
C.E.
C.I.
V.C.A.S.
A.S.
R.E.A. 1.
A.E.A.
Rib.
V.C.P.
G. Blad.
Int.
V.C.P.
Liv.
A.M.C.
V. Sp. S.
V.A.A.
Rect.
V. Ve.
A.L.
V.R.R.
Blad.

FIG. 1.

VII.—*On the Structure and Affinities of* Acmopyle Pancheri, Pilger *

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(*Communicated by Prof.* A. C. Seward, *F.R.S.*)

(Received May 12, 1919,—Read April 29, 1920.)

[Plates 9–11.]

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Introductory.

Our knowledge of the monotypic New Caledonian genus *Acmopyle* is practically confined to incomplete descriptions of the external features by A. Brongniart and Gris (1869), Sir J. D. Hooker (1902), and Pilger (1903). The only other accounts of the plant are brief references in the 'Genera Plantarum' (Bentham and Hooker, 1883), the 'Kew Bulletin' (1892), and 'The Gardeners' Chronicle' (Masters, 1892). I therefore gladly welcomed Prof. Seward's suggestion that I should undertake an investigation of some material collected in New Caledonia by Prof. R. H. Compton, M.A., during his expedition to that island in 1914.† My very sincere

* Thesis approved for the degree of Doctor of Science at the University of London, 1919.

† A preliminary account of this work was read before the Cambridge Philosophical Society on May 20, 1918. See 'Nature,' vol. 101, p. 299.

thanks are due to Prof. COMPTON for his generosity in allowing me to work at this rare material.

To Prof. SEWARD I owe a debt which I cannot adequately express; besides affording me the opportunity for carrying out this research, he showed an unfailing and kindly interest in the work, which, with his advice and able criticism on difficult theoretical questions, was quite indispensable to me. I am also deeply grateful to him for the privilege of consulting the proofs of the fourth volume of his work on Fossil Plants, the publication of which is shortly expected.

New Caledonia shares with many other islands in the Pacific the possession of a large number of endemic species. So far as the Podocarpineæ are concerned, PILGER (1903, p. 36) has already drawn attention to the peculiarities of the New Caledonian flora, which includes at least nine species of Podocarpineæ hitherto recorded only from that island. With the exception of *Acmopyle*, all of these plants are being investigated by Mr. C. P. DUTT, of Queen's College, Cambridge. Mr. DUTT was kind enough to allow me to examine his preparations for comparison—a fact which helped me considerably in my work on *Acmopyle*. In particular, I would like to thank him for allowing me to reproduce in this paper a photograph (Plate 11, fig. 31), of the stone of a new species of *Podocarpus* discovered by Prof. COMPTON in New Caledonia.

The material of *Acmopyle* was originally preserved mostly in formalin, but was subsequently transferred to a mixture of alcohol and glycerine. It consisted of vegetative shoots bearing twigs with pectinately arranged leaves; a stouter piece of stem with about twenty well marked growth rings; male cones in two stages of development; and over a dozen megastrobili. At the time of collection, March 10, 1914, the ovules had all reached the stage when they contain young embryos at the ends of the tortuous suspensor tubes; they had evidently received the last year's pollen. The fact that most of the male cones collected were nearly mature, suggested that younger ovules (in a stage shortly before pollination) would also be present, but there were none such in the material at my disposal. It is hoped that this unfortunate gap will soon be filled up when more material becomes available.

Through the courtesy of the Director I obtained from the Royal Botanic Gardens, Kew, some material of roots and leaves from two young plants growing in the Temperate House. I would also like to thank Mr. L. A. BOODLE for facilities to fix this material at the Jodrell Laboratory.

Besides the two young plants at the Kew Gardens there is another at the Glasnevin Garden, Dublin, from which, through the great kindness of the Director, Sir F. MOORE, I received a fresh twig which has been very useful for comparison.

This research was aided by a Research Studentship at Emmanuel College, Cambridge, and by a grant from the Dixon Fund of the University of London. To both these bodies I am thankful for the financial help.

Historical.

In 1869 A. Brongniart and Gris described under the name *Dacrydium Pancheri* some specimens collected in New Caledonia and sent to them by Pancher. In the notes attached to his specimens the latter had named the plant *Podocarpus pectinata*, a designation which apparently still persists in nurseries. Whereas this name is fully justified by the *Podocarpus*-like habit, and by the form and arrangement of the largest leaves, which are quite distinct from those commonly associated with *Dacrydium*, the position of the micropyle led Brongniart and Gris to transfer the plant to the latter genus. Here it was supposed to occupy a peculiar position, firstly, on account of the drupaceous character of the seed—another feature that justifies Pancher's reference to *Podocarpus*—and, secondly, because of the entire absence, as the joint authors believed, of the structure now generally known as the epimatium. The brief account by Brongniart and Gris, based only on vegetative material and immature seeds, was unaccompanied by figures.

In 1902 Sir J. D. Hooker published, for the first time, figures of a shoot bearing male cones, and of stamens showing the dehiscence of the pollen-sacs. Following the Genera Plantarum he placed the plant in the genus *Podocarpus* (§ Dacrycarpus). Hooker, though unable, in the absence of female flowers and ripe fruit, to fix the generic position of the plant, suggested the possibility that it might prove to be an independent genus.

In 1903 Pilger transferred the plant to a new genus of doubtful affinity, and called it *Acmopyle Pancheri*, the generic name having reference to the position of the micropyle, which, however, is not strictly apical. He was the first to describe the ripe seed, in which the micropyle is according to him hardly visible. In the absence of young ovules he leaves undecided the question as to the existence of an epimatium and, with it, the problem as to whether the entire thickness of the seed-coat is formed by the integument alone. His description is on the whole correct, but the biseriate disposition of the leaves on the terminal shoots, although mentioned in the text, does not appear in the figure. In his dried material, moreover, Pilger appears to have missed the curved ridge at the back of the seed.

Coulter and Chamberlain (1917, p. 353) have recently referred *Acmopyle** to the Taxineæ, thus placing it nearer to *Taxus* than to *Podocarpus*. This is evidently due to oversight, for the little that is known about the plant points clearly to an affinity with *Podocarpus* rather than with *Taxus*.

In the published literature the plant is first mentioned as having been introduced into England in 1891, when the Royal Gardens at Kew ('Kew Bulletin,' p. 105) received a living specimen from the Sydney Botanic Garden. This plant flowered at Kew in 1902, only male flowers being produced, so far as I can ascertain; it is the original of Hooker's figures (1902), the first ever published. Masters (1892) also

* And also *Polypodiopsis*, which has long been known as a synonym of *Podocarpus vitiensis*, A. Brongn. See Bertrand, 1874, p. 65.

drew attention to a plant having been discovered at the Orchid Nurseries of Messrs. Sanders, of St. Albans, England.

Descriptive.

A. *Distribution and Habit.*

Pilger mentions *Acmopyle* as being peculiar to New Caledonia. There is, however, at the Royal Herbarium, Kew, a specimen which although unfortunately sterile, in its vegetative features, external and internal, agrees so closely with *Acmopyle* that it is undoubtedly to be referred to this genus.* The specimen is labelled :

"Fiji Islands, Coll. J. Horne, 1877–78. Received March, 1879,"

but Horne in his book "A Year in Fiji" (1881) does not mention it, possibly because he had no flowers. On the other hand it is, of course, possible that there was an error in labelling the specimen as coming from Fiji. But considering that *Podocarpus elata* R. Br. remained unnoticed in Fiji till 1907 when it was first recorded by Miss L. S. Gibbs† (1909, p. 183) it would not be astonishing if in the absence of flowers *Acmopyle* has escaped detection.

The photograph on Plate 9, fig. 1, is from the older of the two plants at the Royal Gardens at Kew, and was taken with the kind permission of the Assistant Director, Mr. A. W. Hill. Although this plant is only a few feet high, while the tree in its native habitat is said to attain a height of 17–20 metres, it gives some idea of the rather yew-like habit of the dorsi-ventral terminal shoots, with their leaves arranged like the pinnæ of a pectinate leaf—a feature which no doubt suggested the specific name adopted by Pancher. There is a wide variation in the number of leaves on each side of these shoots, and local departures from the pectinate arrangement are frequent; this is especially the case in the shoots bearing the male cones, at least so far as can be judged from the available material. Possibly the male shoots are more often held erect, although this is not the impression given by Hooker's figure (1902).

B. *Root.*

The roots of the Podocarpineæ (except *Pherosphæra* and *Acmopyle*) have formed the subject of a recent paper by Miss Spratt (1913). My material, as already stated, was from the plants growing at Kew, and consisted of young roots up to about 2 mm. thick, with hardly any secondary xylem. As expected, tubercles were present, though only in small numbers. None of them appreciably exceeded the roots in

* I wish to express here my appreciation of Dr. Stapf's kindness in sending me a portion of this specimen, which enabled me to examine it anatomically, and also to thank him for advice concerning the identity of the specimen in question. Dr. Stapf, who kindly examined my sections, does not consider it likely that the Fiji specimen is specifically identical with *Acmopyle Pancheri*, but he agrees to the generic identity.

† I am also much indebted to this lady, who has an intimate knowledge of the flora of Fiji, for her kind advice on the above question.

diameter; several of them had proliferated, having continued their growth distally into normal rootlets (Plate 9, fig. 2), a fact which supports VAN TIEGHEM'S view (1889, p. 352) that the tubercles are modified rootlets. In some material collected at a later date no tubercles were present; this may be due to all the tubercles having grown out into normal rootlets.

The stele of the tubercle, like that of the root, is diarch, and lies in the same plane, at least at its point of origin. Root-hairs were altogether absent in my material, both on the root and tubercles.

In the root the cells of the inner cortex show pronounced local thickenings which, in the form of hoops, girdle the cells in different planes. Similar thickenings have been described by NOELLE (1910) in the Araucarineæ, Taxodineæ and Cupressineæ. In section (Plate 9, fig. 3) these thickenings appear like beads, and turn yellow in SCHULTZE'S solution. EICHLER (1889, p. 34) also describes delicate spiral thickenings in the outer cortical cells in the roots of *Sequoia gigantea*, *Cryptomeria*, *Chamæcyparis*, and in *Podocarpus*, *Phyllocladus* and *Torreya*. The endodermis consists of 2–3 layers of tabular cells which are in radial seriation and give a bright yellow reaction with SCHULTZE'S solution; the outer tangential walls of the outermost layer are much thickened and dark brown. The pericycle, 5–6 layers thick at the sides of the xylem-plate, thins down to 2–3 layers opposite the protoxylems.

The structure of the tubercles presents few features not already noticed by Miss SPRATT in *Podocarpus*. The infected cells lie in an ill-defined zone about halfway out in the cortex (Plate 9, fig. 2); the bars of thickening in the cortical cells are very inconspicuous and sometimes hardly visible, probably because of the young stage of the material.

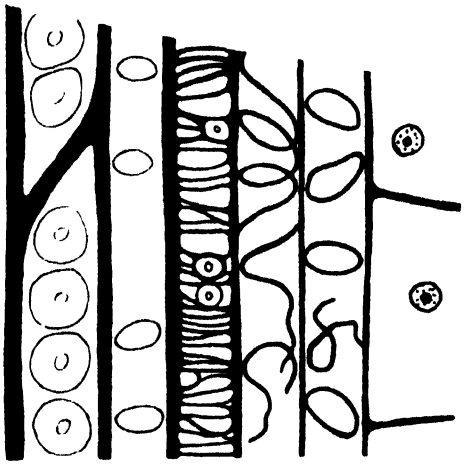
C. *Stem.*

The stem anatomy closely resembles that well known in *Podocarpus*. A section across a young twig before secondary growth shows the usual ring of collateral endarch strands, each with a mucilage canal outside it.* At this stage the pith and cortex cells are all thin-walled, some of them filled with a homogeneous, brown, resinous-looking substance.† In branches a few years old stone-cells appear in the pith and cortex, while the colourless thin-walled cells become starch-laden. In the oldest shoot available (with about twenty growth-rings in the wood), the secondary phloem contains much-elongated fibres which in transverse section are rectangular; they appear exactly like the flattened "autumn" tracheides, and conform to the radial seriation of the xylem and phloem elements. These fibres are comparable

* The contents of these canals become white and opaque in strong alcohol, are slow in taking up stains, show a finely granular or alveolar structure, and usually include deep-staining tabular crystals of different sizes, varying in shape between triangles, squares and hexagons. See fig. 10, Plate 10.

† Although this substance, so commonly present in the Podocarpineæ and other Conifers, usually passes for resin, it is not soluble in alcohol. The only justification for the use of the word resin is convenience of description.

with the sclerites described by TISON (1909, p. 140) in the secondary phloem of *Saxegothæa*. The secondary phloem also contains abundant "resin"-cells, which are arranged in discontinuous rings concentric with the woody cylinder.



TEXT-FIG. 1.

The secondary wood is so similar to that of *Podocarpus* that it is needless to describe it, but attention must be drawn to the sculpturing of the tracheides composing the protoxylem and the metaxylem adjacent to it. Although the narrower protoxylem elements are of the ordinary spiral or annular type, many of the wider ones are scalariform, and also possess circular or oval pits with or without a border, thus closely resembling the transfusion tracheides in the leaf (see below). Some of the earlier-formed metaxylem elements, even in regions not in contact with medullary rays, show large uniseriate borderless pits (Eiporen) at long intervals, instead of the usual bordered pits (text-fig. 1). Exactly the same type of pitting is seen also in the early metaxylem of the leaf-strand.

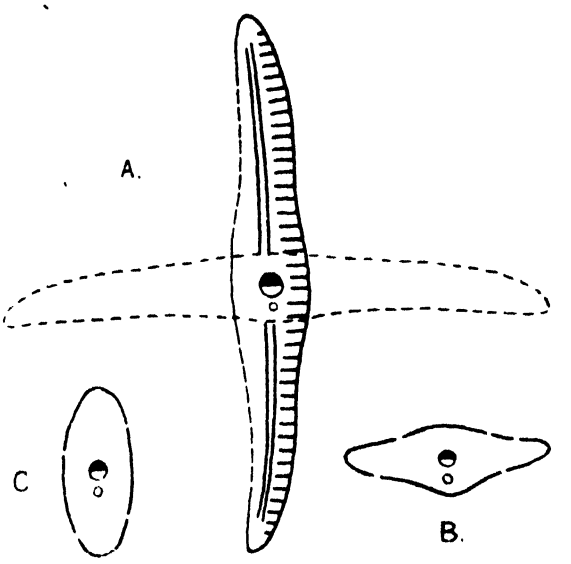
D. *Leaf.*

The polymorphism of the leaf in the Conifers has for a long time drawn the attention of botanists (MASTERS, 1891). In *Acmopyle* at least six types of leaves occur in different regions of the plant :—

1. The large triangular scale-leaves on the thicker branches.
2. The minute imbricate scales on the peduncles of the megastrobili.
3. The chief assimilating leaves, further described below.
4. Small laterally compressed leaves with an isobilateral structure, forming the transitions from 1 to 3 and 3 to 2.
5. The sterile bracts on the fleshy receptacle.
6. The fertile bracts.

The leaves on the dorsi-ventral pectinate shoots deserve special mention, for they afford a striking illustration of the direct influence of the orientation of an organ upon its internal structure. The rather fleshy, linear, obtusely pointed leaves have their smooth and shining, slightly convex, upper surfaces directed towards the light (Plate 11, fig. 22*b*), while the shaded under sides present, on each side of the faint midrib, a broad glaucous band to which the stomata are usually confined, but which is not wide enough to extend to the leaf-edge. A transverse section shows a rather unexpected feature : the plane passing through the xylem, phloem, and the associated mucilage canal, instead of being perpendicular to the leaf surface as in all ordinary leaves, is here parallel to it (Plate 9, fig. 4, and text-fig. 2).

Since there is no sign of a torsion in the decurrent leaf-base it is obvious that the leaf is expanded in the antero-posterior plane, so that the middle lines of the actual upper and lower surfaces represent the margins of an ordinary leaf. The whole anatomy of the leaf, except the vascular strand, has, so to speak, been shifted through an angle of 90°. It is clear, also, that this shifting has taken place in opposite directions in the leaves respectively on the right and left sides of the shoot, for the palisade layer is on the morphologically left side in the right-hand series of leaves, and on the morphologically right side in the left-hand series. As BERNARD (1904, p. 256) points out, leaves thus constituted contradict LIGNIER'S view that the accessory transfusion tissue is the remnant of a former system of veins.



TEXT-FIG. 2.—Diagrams to show the organisation of three different types of leaves in *Acmopyle*. In A the palisade tissue is indicated as short transverse lines, and the accessory transfusion tissue as long parallel lines; the broken line shows the position of the lamina in an ordinary leaf (expanded in the horizontal plane). *Xylem*, black; *phloem*, unshaded.

Towards the base and apex of the pectinate shoot, but especially in the latter region, are frequently seen a few smaller leaves, which indicate the manner in which this peculiar structure has been attained. They show all transitions, both in orientation and anatomical structure, between the linear leaves already described and the spirally placed scale-leaves by which they are distally succeeded. Text-fig. 2, C, shows the lenticular transverse section of one of these transitional leaves, expanded in the radial plane, devoid of palisade cells, and with the stomata evenly distributed on the two faces. Leaves expanded in the radial plane, and having an isobilateral structure, are also met with in the New Caledonian species *Dacrydium taxoides*, and probably in several other Podocarpineæ (see MAHLERT, 1885, p. 279, and VAN TIEGHEM, 1891, pp. 169, 171).

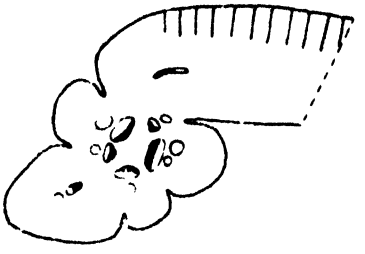
It is well known that, in the horizontal shoots of *Taxus* and many other plants, the apparently distichous arrangement of the leaves is brought about by a torsion in the leaf-bases, some of which at the same time bend round the axis of the shoot, so as to occupy approximately the same (horizontal) plane as the leaves arising from the right and left sides of the axis. In *Acmopyle*, however, no trace of a torsion is seen in any of the leaves; these are all attached by a narrow longitudinally extended base from which the lower edge of the leaf can be followed a considerable distance backwards as a straight decurrent ridge. That here, as in *Taxus*, the leaves arise spirally is seen from the leaf-traces and from the salients caused by the decurrent bases in the outline of a transverse section of the shoot. Since, however, the leaves

are already expanded in the radial plane, a simple bending (unaccompanied by torsion) suffices to bring them into the horizontal position (text-fig. 3), and even this bending is dispensed with in the case of the leaves arising from the sides of the axis.

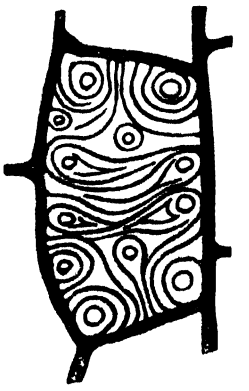
Examples of leaves which show the closest resemblance in principle to those of *Acmopyle* also occur in *Podocarpus imbricatus* Bl. and *P. dacrydioides* A. Rich., a fact noticed in a passing reference by BERNARD (1904, p. 256), but not elucidated by figures.

Prof. GOEBEL has recently (1913, pp. 238–247, 289) described rather similar adaptations in other Conifers (*Thujopsis*, etc.) and some Vascular Cryptogams (*Lycopodium complanatum*, *L. volubile*, *Tmesipteris*). See also BOODLE (1900).

Plate 9, figs. 4–7, and text-fig. 4, illustrate the minute structure of one of the leaves on the pectinate shoot of *Acmopyle*. The thick upper and lower cuticles, the sunken stomata, and the fleshy character of the leaf, are features consistent with the xerophytic habitat of *Acmopyle*. The plant grows, according to PILGER, in rocky places in the south of New Caledonia.* There is the usual palisade layer on the upper side, and the spongy parenchyma below. Sections cut parallel to the leaf surface present a mosaic of large thin-walled mucilage cells, which probably store water, and are responsible for the fleshy nature of the leaf, and groups of much smaller dark-staining palisade cells with abundant interspaces (Plate 9, fig. 5). Hypodermal fibrous elements occur in small numbers on both faces of the leaf, but are confined to the region of the midrib. Between the two mesophylls lies a loose network of transversely elongated cells (Plate 9, fig. 6), several layers thick, extending in the horizontal plane from the median vein to either edge of the leaf. This network consists of (1) thin-walled cells with living contents; (2) dead cells with thick, simply pitted walls ("accessory transfusion tissue," WORSDELL).



TEXT-FIG. 3.



TEXT-FIG. 4.

The transfusion tissue proper, which is as a rule clearly separated from the accessory by a layer or two of parenchyma, is further distinguished by its elements

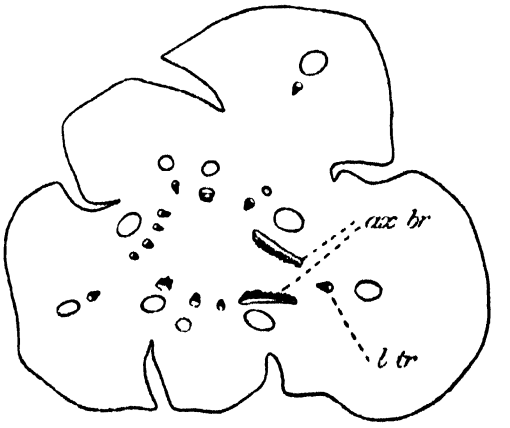
* I have to thank Prof. COMPTON for the following additional information regarding the habitat of *Acmopyle*. The typical situation in which *Acmopyle* grows is in mixed forest containing many species of conifers, on serpentine rocks above 3000 feet altitude. Here it is associated with *Podocarpus minor*, *P. sp.* (unpublished), *Dacrydium taxoides*, *D. lycopodioides*, etc. The majority of arboreal plants in this association show some xerophilous features.

having all their walls provided with crowded bar thickenings, as well as small borderless or narrowly-bordered pits (text-fig. 4). These transfusion tracheides are of two types, one long and narrow, the other considerably broader and nearly isodiametric, but intermediate shapes are also met with. The sculpturing on all is of the same type. The narrower elements are almost all on the side nearest to the protoxylem. In the median plane the transfusion tissue is also separated from the protoxylem by one or two layers of parenchyma, but, laterally, it is often contiguous with the wood. The protoxylem and centrifugal xylem of the leaf show the same types of pitting as described above for the corresponding tissues in the stem.

The epidermal cells, which in the non-stomatiferous regions are longitudinally extended, have straight (as opposed to sinuous) walls, which show moniliform thickenings (Plate 9, fig. 7). The lumina are almost completely filled up by a homogeneous dark brown substance, but the nucleus invariably lies in a pocket in the latter. The stomata are of the usual Gymnospermous type, and the figures on Plate 9, figs. 7*a*–*d*, will suffice to describe them.

E. *The Vascular Supply of the Axillary Branch.*

For reasons stated below, attention is particularly directed to the vascular supply to the axillary branch, which originates as two strands (*ax.br.*), one from each side of the gap in the main cylinder, caused by the subtending leaf-trace (*l.tr.*), (text-fig. 5). Before passing into the branch, the strands turn round, so as to face each other by their xylem ends. It may also be mentioned that this mode of origin is of wide occurrence among Conifers (GEYLER, 1867; STRASBURGER, 1872).



TEXT-FIG. 5.

F. *Microstrobilus.*

The male cones are terminal, and either solitary, or more frequently in groups of 2–5 or 6 (Plate 10, fig. 12), on short peduncles covered with scales. When in a group they are borne on a branched system of peduncles. Each sporophyll bears two spherical abaxial pollen-sacs, except at the base of the cone, where sporophylls with one and three sacs are of frequent occurrence. The longitudinal section given in Plate 9, fig. 8, shows at × the organic apex of the axis, and at ✱ the terminal sporophyll, which is distinctly peltate. The development of all the sporangia in the cone appears to take place almost simultaneously. Neither in the structure of the cone-axis, nor in that of the sporophylls, is there any important difference from *Podocarpus*; a reference to the figures, and the explanations attached to them, will suffice (Plates 9, 10, figs. 8–12).

As in *Podocarpus* and *Dacrydium*, the pollen-grains have two wings, with reticulate markings (Plate 10, fig. 11). Unfortunately, the contents of the pollen-grains are badly shrunken in all the material, so that the structure of the male gametophyte cannot be definitely ascertained. Not more than one nucleus can with certainty be made out in each pollen-grain, but it is highly improbable that no more are produced.

Another feature, perhaps of little significance, in which *Acmopyle* resembles *Podocarpus*, is the thick-walled character of the pollen-tube (STILES, 1912, p. 483, *P. macrophyllus*).

G. *Megastrobilus.*

The field notes accompanying the material are not explicit as to whether *Acmopyle* is monœcious or dicœcious, but the probability is in favour of the latter, for none of the published accounts or figures show both sexes as being represented on the same plant.

External Features.

The megastrobili occur terminally on the pectinate shoots;* as a rule solitary, they sometimes occur in pairs (Plate 11, fig. 22), and, according to BRONGNIART and GRIS (1869, p. 330), even in threes. Each strobilus is borne directly on a long peduncle, and consists of a large fleshy receptacle, ending in a single plum-like seed.† The photographs on Plate 11, figs. 22, 23, are natural size. At the stage represented, the peduncle is strongly curved, sometimes almost into a semicircle; it is completely covered with adpressed scale-leaves, each consisting of a basal cushion adnate to the axis, and a free triangular apex provided with a faint abaxial keel. The peduncle tapers slightly towards the base, for the cushions of the leaves diminish in size, while the keels become more prominent.

The receptacle, sharply marked off from the peduncle by a sudden increase in diameter, is 10–18 mm. long and 8–10 mm. in diameter, being slightly thicker distally. From its remarkably verrucose or tuberculate surface project the thick scale-like tips of a few distant sterile bracts. These bracts are probably in serial continuation with the scales on the peduncle, although their arrangement is obscured by the much greater length and swollen character of the internodes; at the base of the receptacle the sterile bracts are of a size and form transitional to the leaves on the distal part of the peduncle.

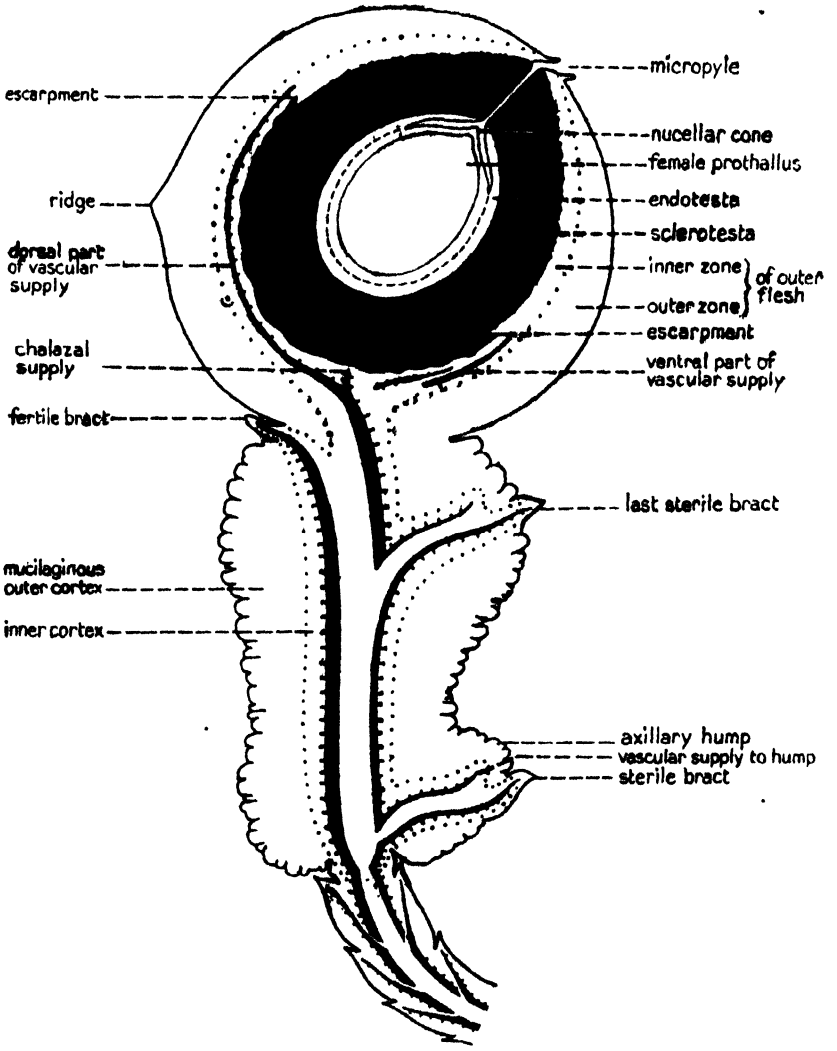
A feature worth noting is that in the axils of many of the sterile bracts the surface of the receptacle is raised into a more or less prominent hump (text-fig. 6), usually distinguishable from the swollen base of the bract. The entire receptacle is thus composed of the swollen axis, the swollen bract bases, and these axillary humps, which, to judge from their vascular supply, are probably vestigial axes (see p. 267).

Attention must be drawn to a curious fact constantly observed in the material at

* PILGER also records strobili in the axils of the distally situated smaller leaves.

† PILGER records 1–2 fertile bracts on each receptacle.

my disposal, namely, that the most distal sterile bract of each receptacle differs from the others in two respects. Firstly, the tip of the bract is awl-shaped instead of scale-like; secondly, the cushion bearing this free tip is considerably larger than the swollen bases of the other bracts, and is always circular with the bract-tip projecting from the centre (Plate 11, fig. 23, text-fig. 6). One face of the receptacle, conve-



TEXT-FIG. 6.—Diagrammatic longitudinal section, in the dorsi-ventral plane, of the peduncle, receptacle, and seed of *Acmopyle* (compare Plate 11, fig. 22*c*). The vascular bundles in the axils of the bracts are shown in the figure, although, being paired, they would not actually be visible in a median section. The *xylem* strands are shown as thick longitudinal lines, the *phloem* as short transverse lines.

niently called the dorsal, is usually free of sterile bracts (Plate 11, fig. 22*a*, is the only exception in my material); this is the side on which is situated, at the distal end of the receptacle, the single, smooth, broadly rotundate, fertile bract (Carpidium, PILGER), easily distinguished from the sterile bracts by its shape and, of course, by the fact that it subtends the seed. In Plate 11, fig. 22*a*, the fertile bract is seen wedged in between the receptacle and the seed. The most distal sterile bract is

always placed on the ventral side of the receptacle, directly, or almost directly opposite to the fertile bract.

Although in the absence of young material a definite opinion cannot be expressed, I rather incline to the view that the large round elevation on the ventral face of the receptacle is the organic apex of the strobilus, pushed aside and overtopped by the developing seed. . The fertile bract would thus be not the most distal leaf on the receptacular axis. This conclusion is based chiefly on analogies in *Podocarpus* and *Dacrydium*. (See especially PILGER'S figure of *D. Bidwillii* (1902, p. 47) and STRASBURGER'S account of *Podocarpus chinensis* (1872, p. 20, and Plate 2, fig. 38)). Miss Gibbs also states (1912, p. 539) that in *P. spicatus* the apex of the strobilus is always distinguishable beyond the last fertile bract.

A similar pushing aside of a primary apex by an enlarging seed has been well known in *Taxus* since VAN TIEGHEM discovered it in 1869 (*loc. cit.*, p. 303, and Plate 16, fig. 91 ; see also STRASBURGER'S well-known figure, 1872, Plate 1, fig. 5).

The unripe seed is an almost perfect sphere, sessile on the apex of the receptacle, with its axis inclined to that of the receptacle at an angle of about 45°. It thus presents a strong contrast to the condition in *Podocarpus*, but approaches that in some species of *Dacrydium*.

The seeds figured by PILGER (1902, p. 117) are all very nearly erect ; this fact may quite possibly be due to his material being slightly older than that examined by me, and may suggest that the young ovule is horizontal or even further inclined, as in some species of *Dacrydium*. However, in the absence of younger material. these remarks can only be taken as conjectures. (See also p. 285, below.) It is worth mentioning that the seeds here described were collected in March, 1914, on Mount Mou.

The surface of the drupaceous seed is covered with bloom and is smooth except for a slight ridge passing obliquely round the base of the seed, but not forming a complete circle. If the seed is viewed from the ventral side (Plate 11, fig. 23), the ridge is hardly visible.

The micropyle is distinctly two-lipped, the upper lip adpressed, the lower projecting and transversely extended.

Anatomy.

The Peduncle.—It was mentioned above that at the stage represented in Plate 11, fig. 22, the peduncle is strongly curved. The plane in which the curvature lies is by no means fixed with regard to the dorsal and ventral faces of the receptacle. In text-fig. 6 it *happens* to coincide with the dorsiventral plane. A section across the distal region of the peduncle shows a striking peculiarity : the strands composing one-half of the vascular ring are much stouter than the rest. This asymmetry was at first suspected to have a relation to the plane of curvature of the peduncle as a mechanical adaptation, either the convex or the concave side having the better developed strands. However, sections through peduncles curved in different planes soon showed that this is not the case : the best developed strands were invariably

found to lie along the line which if produced distally would pass along the dorsal face of the receptacle and through the fertile bract. It will later be seen that this want of symmetry in the vascular system is continued right through the receptacle itself, and that it is no doubt connected with the needs of the developing ovule.

The Receptacle.—(a) *Non-Vascular Tissues.*—The ground-tissue is rather sharply marked off into two concentric regions. The central area comprises the pith, medullary rays, and a few of the inner cortical cell-layers; it is characterised by the small size of the thin-walled longitudinally extended cells, by the entire absence of mucilage cells, and by the presence of small scattered "resin"-cells. The peripheral zone differs in the much larger size of the cells, which, moreover, are mostly radially elongated, and especially in the great preponderance of huge mucilage cells, which form the bulk of the outer cortex, and are responsible for the fleshiness of the receptacle. The large amount of mucilage in the receptacle and in the outer flesh of the seed very probably serves as food to birds, which no doubt disperse the seed. Hand-sections of the receptacle, when placed in water, swell up markedly in their peripheral region, while the central portion remains unaltered. The clear contents of the mucilage cells become white and opaque in strong alcohol; they are slow in taking up stains, and show a granular or alveolar structure. "Resin"-cells are also present in the outer cortex; they are larger and more abundant than in the pith, and, like the mucilage cells, are radially elongated, frequently forming radial chains (Plate 10, fig. 13).

Fig. 13, Plate 10, shows the rather deeply lobed appearance of the tuberculate surface of the receptacle. A specially thick cuticle covers the spherical ends of the lobes, and the small-celled epidermal layer is exceedingly rich in the dark-brown "resinous" matter. No stomata were observed on any part of the receptacle.

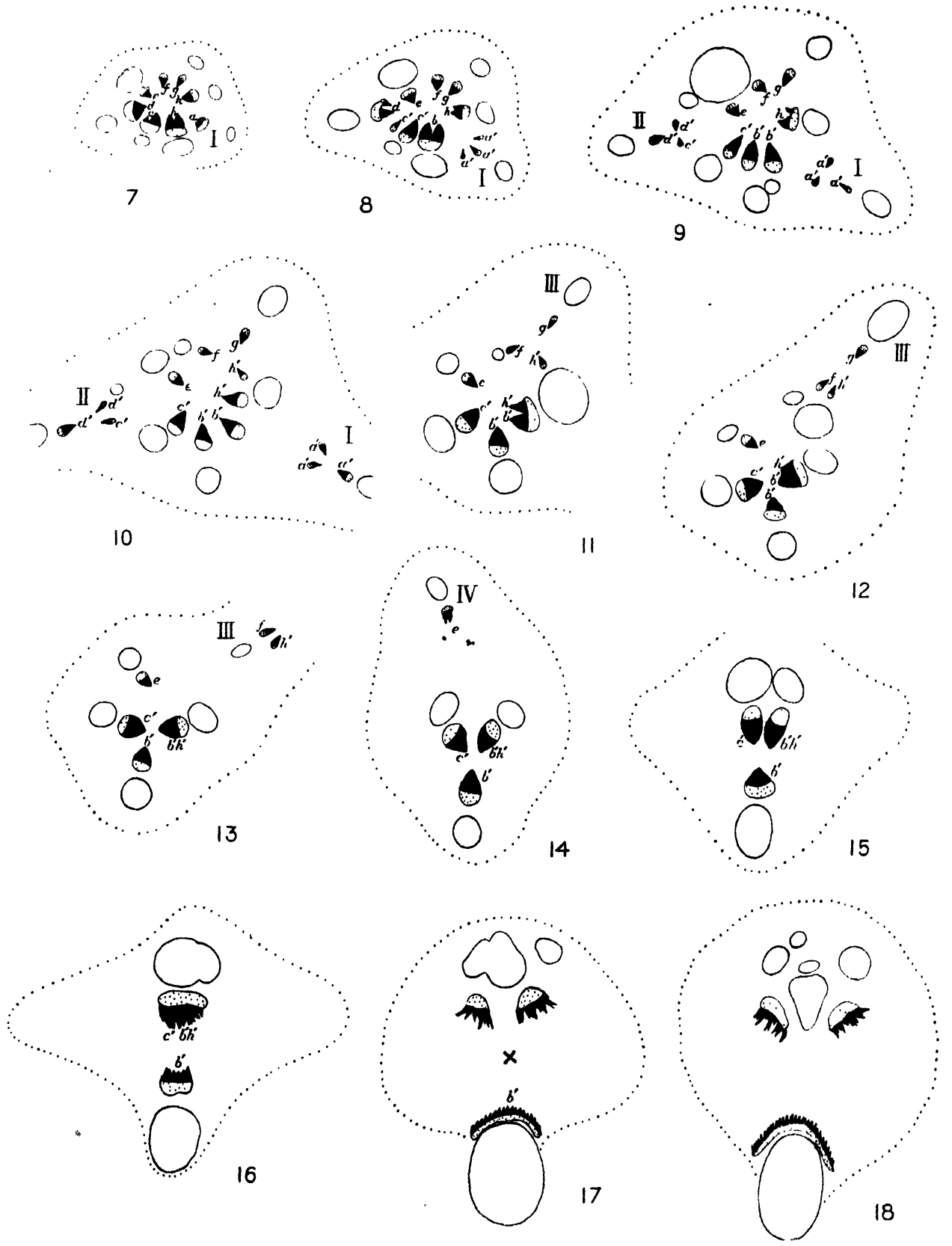
The free tips of the sterile bracts have a thick cuticle perforated by a few stomata on both faces of the bract. In the fertile bract the lower cuticle is considerably thicker than the upper, both being pierced by irregularly distributed stomata.

(b) *Vascular Anatomy of the Receptacle.*—Of all the known Podocarpineæ the vascular system of the receptacle is the most complex in *Acmopyle*. In broad outline it may be described as a dictyostele from which a simple leaf-trace is given off to supply each of the bracts, and in typical cases two strands come off from the sides of the leaf-gap to supply the humps in the axils of the bracts.

The following account is based chiefly on complete series of transverse sections of three whole receptacles and portions of two others. Of these one was embedded in paraffin after most of the cortex had been removed, and was microtomed from base to apex.

A description will first be given of one of the receptacles, then the more important variations observed in the others will be dealt with.

Serial sections cut from the distal end of the peduncle and carried up into the base of the receptacle show at the junction between the two regions a slight constriction in



TEXT-FIGS. 7–18.—Vascular anatomy of the receptacle of *Acmopyle*, as revealed in serial transverse sections from the base upwards. *Xylem*, black; *phloem*, dotted. The peripheral dotted line represents approximately the limit between the inner and outer cortex. In all the figures the dorsal (posterior) side of the receptacle faces the foot of the page. Further explanation in the text.

the pith, which suddenly expands again distally, though not in proportion to the enormous increase in diameter of the receptacle as compared with the peduncle Text-fig. 7, from the base of the receptacle, shows a ring of about eight collateral endarch strands of which those lying on the posterior side are distinctly the largest. Surrounding the strands is a ring of seven mucilage canals, one outside each strand, except for one of the strands which is without a canal.

The receptacle in question had four sterile bracts; three of these had each a well-developed axillary hump; the fourth and most distal arose, as already described, from the summit of a relatively large circular prominence on the ventral face of the receptacle. In the figures the Roman numerals refer to the vascular supplies of these sterile bracts in acropetal order. It is not always easy to speak, in definite terms, of the exact number of strands in the axial cylinder for, as the diagrams show, several of them are in different stages of fusion and disintegration; but it is hoped that the letters adjoining them will enable the reader to identify the strands, or their components at different levels, without unnecessary reference to the text.

Sterile Bract I: Strand *a* comes off bodily from the ring, causing in the latter a gap which closes up in text-fig. 11. The strand immediatelly splits up radially into three pieces which soon arrange themselves fanwise, the canal of the original strand being at the back of the middle piece. This arrangement is brought about by the two lateral pieces turning away from the median with their protoxylem ends as pivots. As we trace the little system obliquely upwards through the cortex of the receptacle the two lateral strands continue their turning movement till they have almost completely reversed their orientation (fig. 10). While the normal median strand with its canal enters the free tip of the sterile bract, there ending in a mass of transfusion tracheides, the two inverted strands end blindly in the mass of small-celled tissue forming the core of the axillary hump. Between the paired inverted strands supplying the axillary hump and those supplying a normal vegetative axillary branch (text-fig. 5) there is such a close resemblance that the hump appears without doubt to be a reduced axillary shoot. See also p. 293 below.

Sterile Bract II: The supply to this bract comes off at only a slightly higher level than that to the first. As before, one of the strands in the ring (*d*) passes out bodily, but in this case it splits up into two unequal pieces (*d'*, *d'*) of which the larger enters the bract-tip. The supply to the axillary hump is in this case formed partly by the smaller piece, and partly by a branch (*c*) from one of the strands of the axial ring. The behaviour of the pair of axillary strands is precisely the same as that already described under Sterile Bract I.

Sterile Bract III: At this stage we have reached a level in the receptacle midway between its base and apex; meanwhile the remaining strands of the ring have undergone considerable re-arrangement which will be clear from the lettering in the figures.

The strand *g* passes out from the ring (fig. 9) and without dividing enters the

bract-tip. The supply to the axillary hump consists as before of two strands (*f*, *h'*). Of these one (*h'*) arises, as in the case of the second bract, by the splitting of one of the strands at the sides of the leaf-gap; the other is constituted by the entire strand (*f*), flanking the other side of the leaf-gap. These two axillary strands behave exactly like those in the axils of Bracts I and II, except that, being appreciably larger than the former, each of them at the same time carries out with it a mucilage canal (fig. 12). These two canals very soon fuse into one (fig. 13).

Sterile Bract IV: We are now near the apex of the receptacle; there is a ring of four strands, three of them much the largest. The smallest of these (*e*), occupying the ventral side of the ring, forms the supply to the fourth sterile bract and its associated hump, which, it will be remembered, is larger than the other humps. The strand passes out undivided through about half the thickness of the cortex, and then gives off from its upper surface two minute strands whose orientation it was not possible to determine (fig. 14). The subsequent behaviour of these strands leaves little doubt as to their homology with the inverted axillary strands above described (see text-fig. 10).

Fertile Bract: There are now three very large strands in the axis (fig. 15). If we trace these strands or their components downwards to the base of the receptacle, we find that the posterior one (*b'*), which is the trace of the fertile bract, has all along preserved its original orientation (text-figs. 7–9). On the other hand, the components of the other two strands (which in fig. 13 face each other, lying in the right-left plane) originally formed part of a more open ring.

As we proceed distally (towards the base of the seed), the two opposing strands continue their turning movement till they come to lie parallel to each other, diametrically opposite both in position and orientation to the third strand. These "inversely orientated" strands compose the vascular supply of the seed; their further behaviour will more appropriately be described later, but attention may be directed to their obvious homology with the paired strands in the axils of the sterile bract-traces. Their relatively huge size is evidently connected with the nutrition of the seed, which may be said to exercise a basipetal influence, which is felt as far back as the peduncle, and results in the asymmetrical development of the entire vascular cylinder.

Shortly before entering the fertile bract, the trace becomes much extended tangentially, and tends to spread round the large mucilage canal, narrow "medullary" rays at the same time breaking it up into a number of thin plates. In the distal part of its course, transfusion tracheides appear in large quantities.

Variations Observed in other Receptacles.—(1) In each of the other two receptacles, which were sectioned from base to apex, the trace of the first sterile bract was not accompanied by any axillary strands.

(2) The last sterile bract of one of these receptacles possessed two unusually large axillary strands, each of which became inversely orientated during its outward

passage, and carried out with it the associated mucilage canal. The two canals did not fuse into one, as described above under Sterile Bract III, but ended blindly shortly before the strands themselves.

(3) The last sterile bract of the other receptacle had only one axillary strand, of medium size, and accompanied by a canal. The behaviour of this single axillary strand was exactly the same as if it were one of a pair, for it did not occupy a median position, and, during its outward course, rotated through an angle of nearly 180°.

In order to check the results obtained from transverse sections, portions of the fourth and fifth receptacles were embedded in paraffin for longitudinal sections, as well as for sections cut transversely to the oblique course of the bract and axillary strands. An axillary strand, as seen in longitudinal section, is shown in text-fig. 6. Turning slightly upwards, in the distal part of its course, it ends blindly in the midst of the small-celled core of the hump.

The Seed. (a) *Non-vascular Structures.*—Text-fig. 6 shows a median section of the unripe seed along the dorsi-ventral plane, which is the only plane of symmetry. The oblique curved ridge on the back of the seed is crossed by this plane about two-fifths of the way up from the fertile bract to the micropyle. The surface of attachment to the receptacle is considerably broader than appears from PILGER's fig. 24*b* (1903, p. 117). PILGER, moreover, figures the seed as being very nearly erect (see his fig. 24*c*); the stony layer is shown proportionately much thicker than it is in any of the seeds examined by me, while the outer flesh is represented as little more than a skin covering the stone, probably because PILGER examined dried material.

The fleshy outer coat of the seed has a roughly uniform thickness of about 2–2·5 mm., except near the apex of the seed. Here it rather abruptly thins to about 0·5 mm., and forms the distal one-third of the micropylar canal. This part of the canal is peculiar in its curved horn-like shape (text-fig. 6), the curvature being in the plane of the section figured. Moreover, this part of the canal traverses the seed-coat very obliquely, being bent at a considerable angle (over 60°), to the straight inner part of the canal, which is bounded by the sclerotesta.

A very thick cuticle forms a smooth protective covering pierced by stomata, which are consequently more deeply sunk than those on the leaf. The distribution of the stomata, which are, as a rule, placed longitudinally, does not show any regularity, except that they are, on the whole, perhaps fewer towards the micropylar end. The lumina of the epidermal cells are small, and nearly always filled with the brown substance above mentioned. At the micropyle the epidermis dips inwards, to line the horn-like part of the canal, the cuticle at the same time gradually becoming thinner.

The fleshy outer coat of the seed is roughly divided into a thick outer zone exceedingly rich in mucilage cells and devoid of vascular tissue, and a thinner

layer immediately covering the sclerotesta. This zone appears much darker than the outer, because it almost entirely consists of "resin"-cells. *The vascular system of the seed is confined to the inner zone.*

The stony layer has a thickness about equal to that of the outer flesh at the seed-base, and encloses a cavity whose diameter almost equals the combined thickness of the stony and outer fleshy layers.

The inner surface of the stone is smooth, but the outer is carved into a pattern which although complex is essentially constant. The photographs on Plate 10 (figs. 15–19) will give a better idea of the sculpturing than will be obtained from a lengthy description. They represent different views of one seed from which most of the flesh was scraped off. The darker lines and patches correspond to the depressions in the surface, from which the remains of the "resinous" inner zone of the flesh could not easily be removed. The surface of the stone is sharply marked off into two regions, an apical and a basal, by a line which may be described as an escarpment facing the micropylar end. (See the sectional view in text-fig. 6.) It will be noticed that while the apical region is characterised by a uniformly granular surface, the basal region shows a series of thick dark lines diverging from the chalaza and ending abruptly at the escarpment. These irregular lines are due to grooves in the stone, and represent the course of the more important vascular strands in the inner region of the outer flesh.

The "escarpment," as seen in the sectional view (text-fig. 6), rather suggests the so-called "crown" which forms the most obvious distinctive feature of the palæozoic seed, *Stephanospermum*. The resemblance is not so clear with *S. akenioides*, Brongn., as with *S. caryoides*, Oliver, where the crown being much less developed, the peri-micropylar trough is shallower (Oliver, 1904, p. 380, text-fig. 2). The comparison is, however, only permissible so far as the longitudinal section is concerned, for while in *Stephanospermum* the edge of the crown forms a complete circle which lies in a single plane transverse to the seed-axis, in *Acmopyle* the line of the escarpment forms two wide loops, one on the ventral face of the stone, the other on the dorsal. These loops, of which the ventral is considerably the deeper, are only narrowly connected together at the micropyle.

It will be seen from text-fig. 6 that the position of the escarpment has no relation either to the ridge on the external surface of the seed, or to the line of separation of the nucellus from the integument.

Prof. Oliver (1903, Plate 24, fig. 13) also figures in *Torreya* a rather similar line on the surface of the stone, but this, as he states (p. 468), marks the line of separation of the integument from the arillus.

According to Sprecher (1907, p. 123, fig. 123), in *Ginkgo biloba* the line of separation of the nucellus and integument is marked by a circular groove on the outer surface of the stone at a level below that of the equator. I am, however, unable to confirm this observation in some material of this plant from Montpellier.

In its symmetry, so far as I know, the stone of *Acmopyle* is unlike that of any other Gymnospermous seed hitherto described. The two faint ridges on the right and left sides (Plate 10, figs. 18, 19), correspond to those on a bicarinate seed of *Ginkgo* or *Taxus*, in which genera they lie in the plane of principal symmetry; but in *Acmopyle* the plane in which the ridges lie is not flat but distinctly curved with the convexity facing the dorsal side (see the side view, Plate 10, fig. 18). An examination of the photographs will show that the chalaza of the stone—if one may employ this term for the point from which the strands radiate—is not situated exactly at the broad end of the stone, so that the straight line joining the micropyle with the chalaza does not represent the greatest length of the stone. The eccentric position of the chalaza is well demonstrated by Plate 10, figs. 15 and 17, which are respectively ventral and basal views. In a view from the dorsal side (fig. 16) the chalaza is not visible. Thus it happens that what in numerous other Gymnospermous seeds corresponds to the plane of principal symmetry, is in *Acmopyle* not a plane of symmetry at all. As is the case with the entire seed, the median dorsi-ventral plane is the only plane of symmetry.

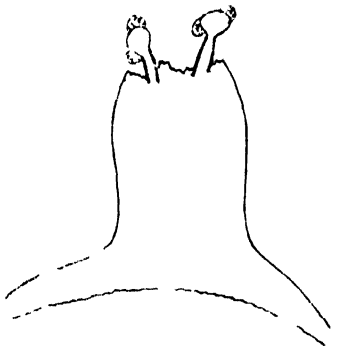
Viewed from either the front or the back the stone has an almost circular outline, there being hardly any trace of a micropylar beak. A section across the middle is isodiametric (Plate 11, figs. 20, 21); the cavity of the stone is here nearly circular, but it is noteworthy that the plane of dehiscence does not traverse it symmetrically, the ventral valve being distinctly larger than the dorsal (text-fig. 32). This asymmetry tends to disappear towards the distal part of the stone. A section across the apical region is lemon-shaped (text-figs. 33, 34), the stone being here slightly flattened in the right-left plane, and produced at each side into a blunt ridge. The portion of the micropylar tube bordered by the sclerotesta is about 2·5 mm. long; throughout this region it is a narrow slit-like passage, flattened in the dorsi-ventral plane (text-fig. 34), and immediately lined by one layer of "resin"-cells continuous with the epidermal lining of the more distal portion of the canal. Meeting this slit at right angles, and joining together the two ridges on the stone are two faint lines representing the plane along which the stone probably dehisces during the germination of the seed.

The cells composing the stone are generally isodiametric, except those along the plane of dehiscence which are flattened in that plane, and those along the cavity of the stone which are flattened tangentially. Their walls are pierced by numerous fine branched canal-like pits which as usual correspond in position on the opposite sides of the middle lamella. In SCHULTZE'S solution the main substance of the wall becomes yellow, but the innermost lining of the cell—which in surface view presents a sieve-like appearance—as well as the lining of the pits, gives the cellulose reaction.

As in *Podocarpus* the inner fleshy layer is for the most part fused to the nucellus (text-fig. 6). The limit between the two is difficult to make out in the fused region, except for the presence, in the inner flesh, of scattered "resin"-cells which increase

in number towards the sclerotesta, and even encroach upon the adjacent regions of the latter. The innermost layer of the free portion of the inner flesh consists largely of "resin"-cells, and is continuous with the rather similar lining of the micropylar canal which may hence be regarded as representing the inner flesh in that region.

The free portion of the nucellus is dome-shaped, and its apex is produced as in *Podocarpus* into a distinct receptive cone or column traversed by several rather thick-walled empty pollen-tubes (text-fig. 36). The nucellar cone, as well as the upper part of the dome upon which the cone is perched, appears white in contrast to the lower part of the dome, whose superficial layer of cells is rich in "resin" (Plate 11, fig. 25). A definite layer of cuticle protects the entire free region of the nucellus except the very tip of the nucellar cone where the cells are crushed and disorganised (text-fig. 36).



TEXT-FIG. 36.—The receptive column of the nucellus of *Acmopyle*, with germinating pollen-grains. The shaded apical portion of the nucellus has become disorganised.

The apex of the female prothallus forms a blunt conical process which lies against the base of the nucellar cone (text-fig. 6 and Plate 3, fig. 26), suggesting the so-called "tent-pole" of *Ginkgo*, *Cordaites*, and other archaic Gymnosperms (BRONGNIART, 1881, Plate 2, fig. 2; Plate 3, fig. 8; Plate 6, fig. 9; Plate 11, fig. 4; Plate 12, fig. 2). The pro-embryo has already been formed; no traces are seen of any aborted archegonia. From the position of the single pro-embryo it may be inferred that the parent archegonium opened a little to one side of the central axis (Plate 11, fig. 26). It may be stated that one prothallus showed an embryo exactly in the axis. In the distended condition of the archegonial wall jacket cells, if there were any in the young archegonium, are not visible.

The structure of the pro-embryo and suspensor tubes presents no features of special interest, being practically identical with that in *Podocarpus*. I am unable to say whether there is a sterile cap to the embryo, such as SINNOTT describes in some species of *Podocarpus* (1913, p. 60).

PILGER (1903, p. 33) records unusually long suspensor tubes in some species of *Podocarpus* (*P. amarus*, 30 mm.). In *Acmopyle* the tubes when stretched out reach a length of over 15 mm.

As in most species of *Podocarpus*, too, the megaspore membrane is poorly developed (Plate 11, fig. 28), although it is possible to distinguish two layers, of which the outer is a film of cuticle. On the conical apex of the megaspore the membrane is particularly thin, the cuticle being absent, or at any rate extremely thin.

Plate 11, fig. 28, shows a longitudinal section of the superficial region of the female

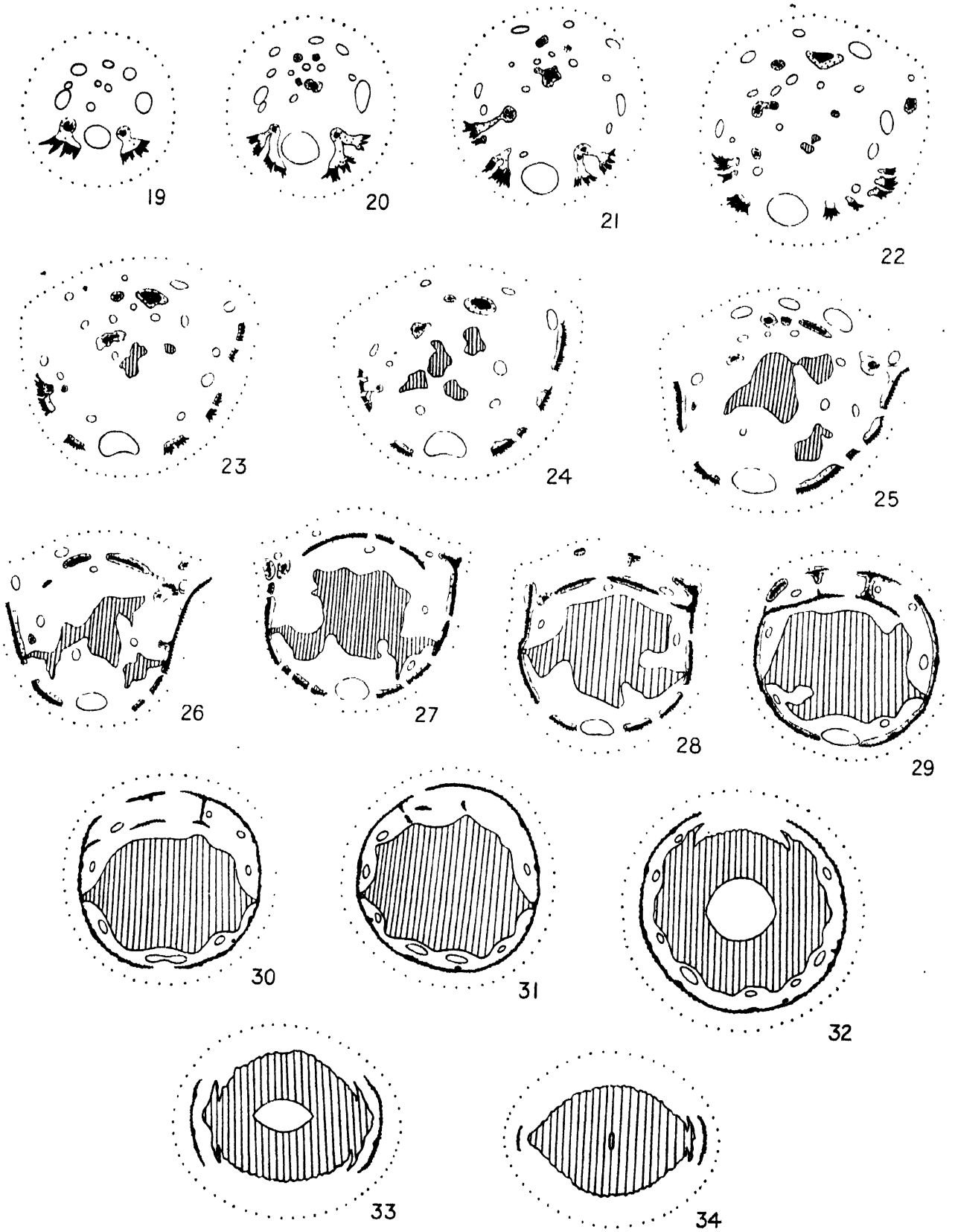
prothallus; the small multi-nucleate cells (see Plate 11, fig. 29) composing the peripheral layer of the prothallus have their anticlinal walls slightly thickened in the regions adjacent to the megaspore membrane. In the last-mentioned figure, which is a surface view of this layer, these walls are seen traversed by straight pits.

All the remaining cells of the prothallus are thin-walled and most of them are multi-nucleate; those forming the axis of the prothallus in front of the advancing embryo are distinguished by their small size, elongated shape and abundant starch-content. No tracheides were seen such as those described by Miss GIBBS (1912, p. 546, Plate 51, figs. 42–3) in *Podocarpus polystachya* and by COKER (1902, p. 97) in *P. coriacea.*

(b) *Vascular Anatomy of the Seed.*—In broad outline the vascular structure of the seed may be described as a tracheal cupule (Plate 11, fig. 24) stiffened by a network of strands and exactly covering the basal region of the stone which, as already mentioned, is limited distally by the "escarpment." A vascular system internal to the stone is not present, but besides two concentric strands terminating against the chalazal end of the stone, a number of other strands come off at different levels from the inner surface of the cupule, and end before reaching the sclerotesta. In the right-left plane the tracheides of the cupule are for some distance in organic connection with the stone cells.

The vascular system will now be described in detail (text-figs. 19–34). Text-fig. 35 shows the approximate levels at which the sections drawn in text-figs. 19–34 were cut. Serial transverse sections were cut of two seeds, and the results confirmed by dissections and longitudinal sections of two others. A reference to fig. 15, p. 266, will show that at the apex of the receptacle there were two very large strands lying parallel to each other and with an orientation inverse to that of the fertile bract-trace. We shall now examine the further behaviour of these strands, for it is chiefly by the disintegration of these that is produced one of the most complex vascular systems known among Gymnospermous seeds.

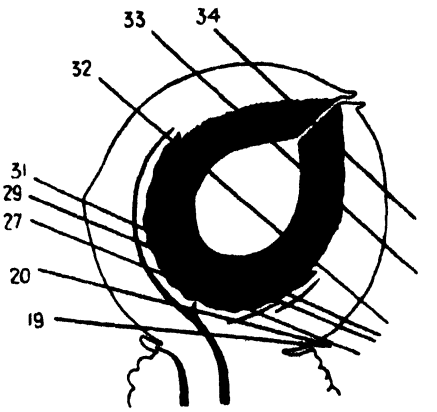
Immediately above the level where we left them on p. 268 these two strands completely fuse together, xylem with xylem and phloem with phloem, while their mucilage canals may also fuse into one (text-fig. 16). However, the resulting large strand immediately breaks up again into two (text-fig. 17); at the same time the two mucilage canals (or one in case of fusion) are replaced by several canals of different sizes which lie in two roughly concentric rings (text-fig. 19). At this level an important change has taken place in the aspect of the cross-section; whereas at first the centre of the figure was towards the xylem side of the strands (at × in fig. 17) it has now shifted suddenly to the phloem side, and lies in the middle of the group of mucilage canals (fig. 19). The cells among which the canals lie are distinguished from the surrounding tissue by their small size, and by the fact that they are nearly all filled with "resin." We are now at the chalazal end of the seed, and these cells constitute the dark internal zone of the fleshy seed-coat, of which the outer limit is



TEXT-FIGS. 19–34.—Vascular anatomy of the seed of *Acmopyle*, as revealed in serial transverse sections. *Xylem*, black; *phloem*, dotted. The peripheral dotted line represents the limit between the two zones of the outer flesh. In all the figures the dorsal (posterior) side of the seed faces the foot of the page. *For the approximate levels at which the different sections were cut, see the corresponding numbers in text-fig.* 35.

marked in the figures by means of a dotted line. Nevertheless, it must be made clear that the boundary is by no means a sharp one.

As we trace the two strands upwards, they diverge from each other, especially by their xylem ends, and at the same time become tangentially extended. One or more radial splits soon appear in the xylem, so that for a time each of them consists of an unbroken mass of phloem facing the centre, and a forked mass of xylem directed outwards.



TEXT-FIG. 35.—Index-figure to text-figs. 19–34.

At about this stage two fairly large concentric strands come off, one from the inner face of each, but they soon dwindle in size, and, before they have proceeded very far towards the centre, they end blindly near the chalazal end of the stone. They are probably the reduced homologues of the two strands in *Ginkgo* which, lying in the principal plane of the seed, penetrate the base of the sclerotesta.

The splits in the xylem of the two large original strands rapidly extend to the phloem, and result in the formation of several distinct strands, which in turn become tangentially flattened, and split up radially into a large number. These tend to envelop the central tissue, including the mucilage canals, amongst which by now the first traces of the stony layer have also appeared (text-figs. 22, 23). This process of fragmentation, accompanied by tangential flattening of the strands, ultimately gives rise to the thin but nearly continuous vascular arc, which, at the level shown in text-fig. 27, covers the dorsal two-thirds of the cross-section.

The similar arc forming the ventral one-third of the vascular ring in the same figure has originated independently of the dorsal arc. In order to trace its mode of origin, we must again refer to the series of sections in text-figs. 19–27. In text-fig. 20 it will be noticed that there is, besides the large dorsal strands, a group of small mostly concentric strands on the ventral side of the section. In the small amount of material examined I could not trace any connection at the chalazal end between the dorsal and the ventral groups. As this fact was subsequently found to be of some theoretical importance (see p. 283, below) in the interpretation of the seed of *Acmopyle*, it was considered worth while to confirm it by means of paraffin sections of the basal region of a third seed (in addition to the two sectioned by hand); no trace of a connection was observed. The ventral strands are in two batches, one inside the inner ring of canals, the other in the space between the two rings. Those of the inner batch terminate after only a short upward course; those of the outer batch, on the contrary, increase in number, and, becoming markedly flattened in the tangential direction, ultimately form the ventral arc seen in text-fig. 27.

Thus, at the level of the figure just referred to, the stone is almost completely

enveloped by two vascular arcs. The ends of the dorsal arc become distinctly curved outwards (text-figs. 26–28), and it is noteworthy that these reflexed ends correspond in position to the ridge on the outer surface of the seed. The dark internal zone of the fleshy seed-coat also shows distinct bulges over these curved ends. So far as I could ascertain, no other disturbance is caused by the ridge in the internal structure of the seed.

As we proceed distally, new strands make their appearance outside the ventral arc, and these establish connections both internally with the ventral arc and laterally with the free ends of the dorsal arc (text-figs. 28–30). For a short distance upwards there are thus two interrupted ventral arcs, which are connected together by radial commissures, and which at their ends converge into the single dorsal arc (text-figs. 29–30). The inner ventral arc now rapidly breaks up and dies out, while the outer at the same time becomes more substantial, with the result that the stone is soon enveloped by a single, practically unbroken, vascular ring (text-fig. 31). In this ring the local thickenings are the transverse sections of the compressed, ill-defined strands, which may be said to form a supporting network for the vascular cupule shown in surface view in Plate 11, fig. 24.

The figure just referred to is from a slightly enlarged photograph of the left half of a seed, from which the outer portion of the fleshy seed-coat was carefully shaved off, so as to expose the tracheal cupule. The photograph was taken with the seed immersed in alcohol, for the cupule then assumed a white colour, and stood out clearly against the dark inner zone of the flesh. At the base are the two large strands forming the main supply. A comparison with the side view (Plate 10, fig. 18) of the naked stone shows that the edge of the cupule closely follows the line of the escarpment. This correspondence is well seen in the sections shown in text-figs. 32–34. As we proceed towards the distal end of the seed, two successive gaps appear in the cupule: first, a gap corresponding to the ventral (deeper) loop of the escarpment (text-fig. 32), and, at a higher level, another corresponding to the dorsal loop (text-fig. 33). The gaps rapidly enlarge, so as to reduce the cupule to two lobes, opposite to the right and left ridges of the stone. These lobes extend up to within a short distance of the micropyle, becoming narrower and narrower as they approach the apex of the seed (text-fig. 34).

At various levels the inner surface of the vascular envelope gives off minute strands towards the sclerotesta, but all of these die out before actually reaching it. Plate 11, fig. 21, is a photograph of a section cut at about the level of text-fig. 32.

Text-figs. 26–33 show a feature worthy of special note, whose importance will be seen in the theoretical part of the paper (p. 286): along two lines which lie in the right-left plane of the seed, the cells composing the stone are for some distance in intimate contact with the tracheides of the cupule. This liaison between the vascular system and the sclerotesta, coupled with the porous structure of the latter, suggests that the sclerotesta may to some extent serve to keep up a supply of water to the

developing female prothallus and embryo. This view seems all the more probable in the absence of a vascular system internal to the sclerotesta. In *Ginkgo*, where there is no vascular system outside the sclerotesta, SPRECHER (1907, p. 137, fig. 150) has drawn attention to the remarkably tracheide-like character of the superficial cells of that layer.

Fig. 27, Plate 11, gives some idea of the different shapes and sizes of the tracheides composing the cupule. While the majority of the tracheides are long, narrow, and generally placed lengthwise, some are broader and nearly isodiametric. The sculpturing is difficult to describe, being of various types grading into each other, but the general impression gained is that of the transfusion tissue of the leaf. Special mention may be made of the occurrence of spiral tracheides, some of which have several bands crossing each other; minute round pits may also be present on these, as they may be on all the remaining types of tracheides composing the vascular investment.

Before closing the description of the seed, it must be stated that in the vascular cupule the position of the phloem with regard to the tracheides could not always be determined with certainty, and that for this reason it was considered best to omit the phloem altogether from some of the drawings (text-figs. 30–34). In text-figs. 28, 29, too, the amphi-phloic nature of the ventral strands is by no means certain. The exceedingly hard sclerotesta makes thin sections impossible, while the preponderance of "resin"-cells tends to obscure the vascular tissues embedded among them.



TEXT-FIG. 37.—Part of transverse section of vascular cupule from the seed of *Acmopyle*. The outer face of the cupule corresponds to the lower edge of the figure. The thick-walled cells are tracheides, the thin-walled cells phloem, the latter being mostly internal to the xylem. In the middle of the figure some tracheides are coming off from the inner face of the cupule. Sculpturing of tracheides not shown.

It appears likely, however, that the major portion at least of the cupule has only an internal lining of phloem—a condition which would be in harmony with the inverted orientation of the ovular supply in *Podocarpus* (see text-fig. 37).

Finally, it may be noted that the mucilage canals—one of which elsewhere in the plant generally accompanies each strand—in the seed have a distribution which is at present not reducible to a definite principle, except that in the distal half of the seed, at least, they all lie in a ring between the sclerotesta and the cupule. Their position

inside the cupule is in conformity with their usual association with the phloem side of strands.

Theoretical.

From the foregoing description of *Acmopyle* it will be agreed that the plant shows some obvious resemblances to *Podocarpus*, the genus to which the discoverer, PANCHER, originally assigned it. The points of resemblance are indeed so numerous and far-reaching that to some systematists it may even appear doubtful whether a generic separation is justified.

Thus the habit, as well as the stem and root structure, including the wood-anatomy and the presence of root-nodules characteristic of the family, are features common to the two genera. The intimate correspondence shown by the two genera in the habit and structure of the male cones and microsporophylls, as well as in the outward form of the pollen-grain, fully justifies the presumption that the same will be found to be the case with the male gametophyte, an important structure which shows, so far as known, a remarkable constancy of plan throughout the Podocarpineæ. In the female organs, too, the thick fleshy receptacle, and especially the drupaceous seed, with the semi-inferior nucellus and the structure of the pro-embryo, find their exact counterparts in *Podocarpus*.

Although the receptacle of *Acmopyle* shows the most complex organisation known among the Podocarps, it does not present a single feature of importance not also met with in *Podocarpus;* and in this respect, so far as I have been able to ascertain, the nearest approach to *Acmopyle* is seen in the § *Dacrycarpus*. On a minute scale, the receptacle of *P. imbricatus*, Bl. (= *P. cupressinus*, R. Br.) recalls that of *Acmopyle*, both in the verrucose or tuberculate surface (Plate 11, fig. 30) and in the vascular anatomy.*

There is at the base a vascular cylinder composed of strands which as in *Acmopyle* are best developed on the side from which at a higher level the ovular supply originates. Paired axillary strands are also associated with some of the spirally disposed bract-traces; the members of each pair after their departure from the axial cylinder execute the familiar rotatory movement, but before completely reversing their orientation end blindly in the axil of the subtending bract. Strands which are no doubt homologous with these have been observed also in *P. spinulosus* (BROOKS and STILES, 1910, Plate 21, fig. 12); in *P. sinensis* (VAN TIEGHEM, 1869, Plate 15, fig. 82); and an unnamed species of *Eupodocarpus* (AASE, 1915, p. 303, fig. 173).

Here we may also mention that the superficial sculpturing of the stone of *Acmopyle* is very similar in pattern to that in a hitherto unpublished New Caledonian species of *Podocarpus* discovered by Prof. R. H. COMPTON. The photograph on Plate 11, fig. 31, will show that, as in *Acmopyle*, the surface of the stone is divided by an escarpment into two areas, although in this case the loops of the escarpment are

* To judge from herbarium specimens and published figures of the external features, *P. dacrydioides* would probably be essentially similar.

deeper than in *Acmopyle*. In a deep-lying structure a resemblance of this kind, apparently of no biological significance to the plant, cannot easily be explained away on the view of parallel development.

Next to *Podocarpus*, *Dacrydium* is the genus which most nearly approaches *Acmopyle*, for several of the above-mentioned *Podocarpus*-like features of *Acmopyle* are also met with in *Dacrydium*. The most obvious point in which the two last-named genera agree is, of course, the sub-erect position of the seed, which is at the same time a notable difference from *Podocarpus*, and it is significant that BRONGNIART and GRIS regarded *Acmopyle* as a species of *Dacrydium*.

Only three characters stand out prominently in *Acmopyle* as being distinct from *Podocarpus*. But they are all of sufficient importance to deserve careful attention, for upon their interpretation largely depends the position of *Acmopyle* within the family :—

Firstly, the vascular structure of the seed is peculiar.

Secondly, the ultimate posture of the ovule is different from that in *Podocarpus*.

Thirdly, the rather perplexing fact that there is, to all appearance at least, no sign of an epimatium.

These points will now be discussed in some detail.

A. *The Interpretation of the Structure of* Acmopyle *in Relation to its Affinities.*

1. *The Epimatium.*

The reader will have noticed that in the descriptive section of this paper no mention was made of an epimatium. This was not because an epimatium is lacking in *Acmopyle*, but because it does not figure as a distinct structure and, consequently, its existence is only inferred from a comparison with related genera. The obvious resemblance to *Podocarpus* and *Dacrydium* naturally lead one to seek a place for *Acmopyle* in the vicinity of those genera, and there is no escape from the conclusion that an epimatium must be present in this genus ; the real problem is to define its limits.

Before we attempt this in the case of *Acmopyle* it is necessary to be clear as to the limits of the epimatium in *Podocarpus* itself. It is well known that in a median longitudinal section (see Plate 11, fig. 30) the ovule of *Podocarpus* shows on the dorsal* side, near the micropyle, a distinct lobe (the free portion of the epimatium), of which there is no counterpart on the ventral (fused) side. The question arises as to whether :

(*a*) The " ovular stalk," adnate to the ventral side of the ovule, should be regarded

* To avoid confusion, it may here be stated that if the ovule of *Podocarpus* is conceived of as being adnate by its entire length to a stalk, the ventral face of the ovule is naturally understood to be that by which the ovule is fused to the stalk. This terminology is consistent with that applied above in describing *Acmopyle*, and may be extended to the other Conifers with inclined or inverted ovules, and even to the anatropous ovules of Angiosperms.

as a part of the epimatium, and the latter therefore as completely surrounding the ovule (PILGER, 1903, p. 16 ; TISON, 1909, p. 147 ; SINNOTT, 1913, p. 41 ; GIBBS, 1912, p. 527 ; STILES, 1912, p. 472) ; or

(*b*) The "ovular stalk" should be regarded as a structure distinct from the epimatium, in which case the latter does not completely surround the ovule.

On the first view the epimatium is homologous with the ovuliferous scale of other conifers ; on the second view only the "ovular stalk" with its distal continuation the "apical knob" (SINNOTT) is homologous with the ovuliferous scale. It is not easy to choose between the two views. In the absence of a histological differentiation between the stalk and the epimatium (in the restricted sense), it would be simpler to regard the entire structure in question as an ovuliferous scale whose margins have become arched over the seed so as completely to enclose the latter. But for convenience of description it is here considered preferable to employ the term epimatium in the restricted sense.*

While it may be immaterial whether the epimatium is regarded as completely surrounding the ovule, it is of real importance to have some idea as to the boundary between the integument and the epimatium which overlies it. The importance of this enquiry becomes apparent when we realise that STILES regards the vascular system of the seed of *Podocarpus* as being entirely epimatial rather than (in part at least) integumental, and that he denies its homology with the vascular system of the other Gymnosperms (STILES, 1912, p. 477).

Since this question is intimately connected with the morphological status of the vascular supply, the full discussion will be given at a later page. Here I will merely state that I consider it more natural to regard the inner part of the outer flesh, which contains all the descending strands, as an integral part of the integument. The epimatium of *Podocarpus* is on this view only the peripheral portion of the flesh and contains no vascular supply.

We are now in a position to attempt an interpretation of the seed of *Acmopyle* in terms of that of *Podocarpus*, its nearest ally. Two views present themselves ; one of them tends to relate *Acmopyle* more to *Dacrydium*, the other to *Podocarpus* :—

(*a*) The epimatium exists in the form of a broad concave scale, similar in shape and position to the epimatium in most species of *Dacrydium* (that is, forming a small asymmetrical cup at the base of the seed), but different in being fused to the integument by its entire inner surface. Its presence is outwardly indicated only by its margin, which forms the ridge (see Plate 11, fig. 22*a*) obliquely girdling the seed-base.

* This restricted use of the term epimatium would, indeed, be a necessity, if it is shown that the close analogy between the ovule of *Podocarpus* and the anatropous ovule of the Angiosperms is based on a homology of the different parts. For, the outer integument of the angiospermous ovule (which would then be homologous with the epimatium), is naturally regarded as a structure distinct from the funiculus, being frequently distinguishable also on the ventral side of the ovule, between the funiculus and the inner integument.

The semi-erect posture of the seed, which may be regarded as an important point of contact with *Dacrydium*, lends countenance to this view, and in virtue of its several *Podocarpus*-like features, *Acmopyle* deserves an independent generic position, bridging the gap between *Dacrydium* and *Podocarpus*. It is significant in connection with this view, that BRONGNIART and GRIS (1869, p. 330), regarded the plant as the only species of *Dacrydium* provided with a drupaceous seed but devoid of an epimatium.

(*b*) The epimatium forms an almost complete investment to the ovule, as in *Podocarpus*, but differs in being entirely fused with the integument, even in the region of the micropyle, in the formation of which it takes part. Thus, as in *Podocarpus*, the "outer flesh" of the seed consists peripherally of the epimatium and internally of the sarcotesta, an integral part of the integument.

The ovular stalk is here represented by the broad concave scale, of which the distal margin forms the curved ridge passing obliquely round the base of the seed. This ridge corresponds to the "apical knob" in *Podocarpus*. Except for the greater fusion of the epimatium *Acmopyle* is practically a *Podocarpus* with a semi-erect and highly vascular seed. So far as the epimatium is concerned the three genera form a linear series with *Podocarpus* in the middle.

The second view is preferable on all hands; it does not involve the assumption of any new morphological combination but fits in with the conditions already existing in *Podocarpus*, the differences being to a large extent traceable to the orientation of the seed. Moreover, the persistence with which the so-called "apical knob" (SINNOTT, 1913, p. 42) occurs both in *Dacrydium* and *Podocarpus* leads to the suspicion that it carries a deep morphological significance; and it is difficult to set aside the obvious similarity of the curved ridge on the back of the seed in *Acmopyle* to that described by Miss GIBBS in *P. vitiensis* (1912, fig. 14). This is no doubt the same structure which in *D. taxoides* (PILGER, 1903, p. 47, fig. 4),* in *D. falciforme* (GIBBS, 1914, p. 192, fig. 8) and in *P. andinus* (PILGER, 1903, p. 65, fig. 10) develops into a long horn-like process, especially in the young ovule, but which in *P. Nagi* and § *Eupodocarpus* is either reduced to an insignificant tip or is completely merged in the scale. There is no doubt, too, that this organ is the homologue of the distal end of the ovuliferous scale of other Conifers (see SINNOTT, 1913, p. 75).

2. *The Orientation of the Seed.*

With its erect seed *Acmopyle* clearly resembles *Dacrydium* rather than *Podocarpus*, and the question arises whether this feature has been retained from a *Dacrydium*-like ancestor, or whether it is a secondary acquirement.

In *Dacrydium* the final position of the ovule varies from the nearly erect (*D. Fonkii*, *D. laxifolium*, etc., see PILGER, 1903, p. 49, fig. 5) to the nearly

* As Miss GIBBS points out (1914, p. 192), PILGER'S figs., H-L, on p. 47 of his 'Monograph,' are erroneously referred to *D. falciforme*.

horizontal (*D. taxoides*, see PILGER, 1903, p. 47, fig. 4) while the completely inverted seed is confined to *Podocarpus*.* SINNOTT (1913, p. 71), who has propounded the reduction theory of Podocarpineæ, regards the inverted seed of *Podocarpus* as the more primitive condition, from which he derives the more erect seed of most species of *Dacrydium*. In accordance with this view he regards as a case of recapitulation the resemblance between the final stage of *Podocarpus* and the young stage of *D. colensoi* and *D. intermedium*, in which the ovule is inverted and the epimatium overtops the integument (SINNOTT, p. 49).†

However, it has long been known through the work of STRASBURGER (1872, Plate 2, figs. 39*ff*) and of BAILLON (1873, Plate 8) that the ovule of *Podocarpus chinensis* executes during development a movement in the opposite direction, namely, from a nearly horizontal to a completely inverted position. STILES (1912, p. 470) records a similar movement in *P. latifolius*, and Miss GIBBS' photograph (1912, fig. 79) of a comparatively young ovule of *P. elata* leads to the same conclusion.

These facts about *Podocarpus* are not here cited as reminiscences of a dacrydioid ancestry; but they at least indicate the uncertainty attending the interpretation of the juvenile stage of *Dacrydium*.

As regards the posture of the young ovule of *Acmopyle* a definite statement is naturally at present out of the question, but even if it is found to be inverted, it would be difficult to argue that the sub-erect position of the ripe seed is the expression of a dacrydioid affinity, for in the only other points in which *Acmopyle* departs from *Podocarpus* it does so in a direction opposite to that of *Dacrydium*. As already concluded above from a consideration of the epimatium the three genera probably form a linear series with *Podocarpus* in the middle; the nearly erect position of the seed of *Acmopyle* is therefore probably to be regarded as a recent secondary acquirement. If the reduction theory is to be maintained—a view to which I do not subscribe—*Acmopyle* must be the most primitive member of the family, both in view of the total fusion of the epimatium and of the great complexity of the entire female reproductive organs.

3. *The Vascular Structure of the Seed.*

This complexity is perhaps most clearly seen in the vascular supply of the seed, which is the best developed not only among the Podocarps but among all known Conifers.

* PILGER states that the three New Zealand species of *Dacrydium* (*D. Bidwillii*, *D. Kirkii*, *D. biforme*) have their seeds completely inverted, as in *Podocarpus*; they have for this reason been regarded as showing an interesting transition between the latter genus and the remaining species of *Dacrydium* (SINNOTT, 1913). I have, however, found evidence to show that *D. Bidwillii*, and probably also the other two species, belong properly to the genus *Podocarpus*, of which they would probably form a distinct section. (SAHNI, 1918A, p. 219.)

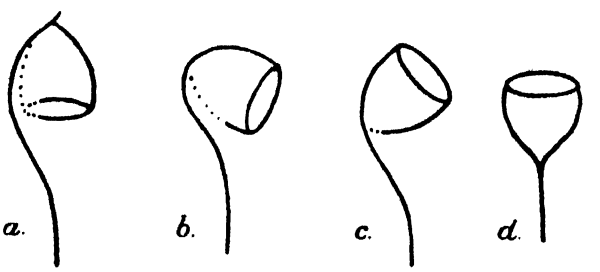
† The same inverted condition is seen in the young ovules of *D. cupressinum* (PILGER, 1903, p. 54, fig. 6), *D. taxoides* (PILGER, p. 47, fig. 4), and *D. falciforme* (GIBBS, 1914, p. 192, fig. 8); on the other hand, some young ovules of *D. laxifolium*, on which I hope soon to publish notes, are only slightly inclined from the erect position, the ripe ovules being perfectly erect.

Apart from the presence in *Acmopyle* of a more or less continuous vascular network (cupule) in place of the separate strands of *Podocarpus*—which is only a matter of degree of development—the differences in the distribution of the vascular supply in the two genera are no doubt to be traced chiefly to the different posture of the seed. This view is illustrated in text-fig. 38 by means of a series of purely diagrammatic sketches of the vascular skeleton. For the sake of simplicity, the descending portion of the ovular supply in *Podocarpus* (text-fig. 38, *a*) is also shown as a continuous vascular mantle, as in *Acmopyle* (text-fig. 38, *c*).

The series of diagrams may be read in either direction according to the view adopted as to the relative antiquity of the two genera. Assuming for the sake of argument, that the ovule of *Podocarpus* is more primitive than that of *Acmopyle*, as the ovule gradually erects itself, its ventral surface would become free from the "ovular stalk," and would thus be left with a gap in the vascular supply originally occupied by the ascending strands. We could then reasonably expect that the existing strands would tend to distribute themselves more evenly round the circumference of the ovule wherever the latter is free from the "stalk," so as to ensure a supply to the ventral side as well. This ventral completion of the vascular ring would begin at the micropylar end (which would be the first to become free) and gradually extend towards the chalaza as the ovule was tilted up. At an inclination of about 45° a condition would be reached not very unlike that actually seen in *Acmopyle*. It is now easy to see the significance of the fact—which at first was rather puzzling—that there is no direct connection, at the chalazal end of the seed of *Acmopyle*, between the strands on the ventral side and those on the dorsal (see p. 275 and text-fig. 20).

The next stage, shown in text-fig. 38, *d*, is hypothetical, and shows the presence of such a connection in the final phase of erection. The stage shown in text-fig. 38, *b*, is also hypothetical.

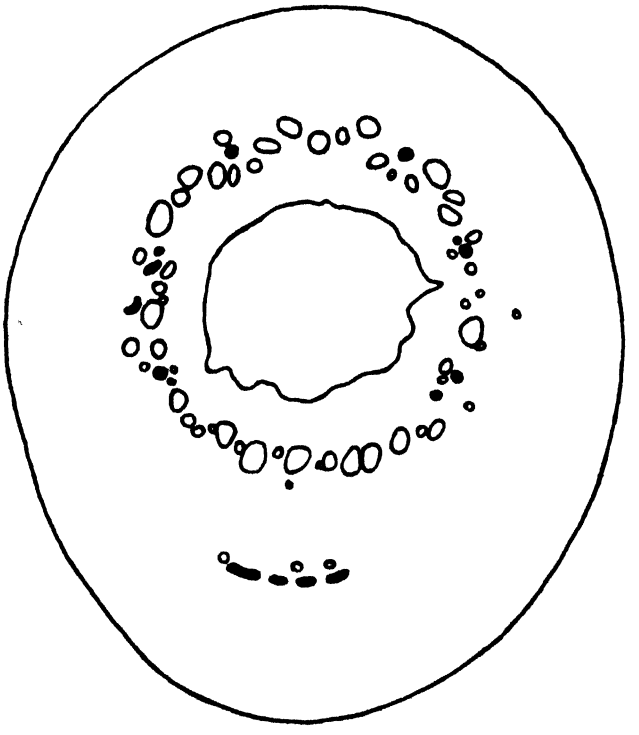
Arguing in the reverse direction—that is, if the seed of *Acmopyle* is regarded as more primitive than that of *Podocarpus*—one would expect that, as the ovule gradually became more and more inclined and its mid-ventral surface became fused to the "stalk," the vascular strands on that side would tend to become atrophied, for they would be superfluous in the proximity of the ascending strands of the "stalk." This atrophy would naturally begin at the chalazal end, and gradually work its way towards the micropyle.



TEXT-FIG. 38.—Diagrams. See text above.

If the above interpretation is correct, it is apparent that in *Podocarpus* the ascending and the descending strands do not properly belong to one and the same system, for the descending strands (which I think belong strictly to the integument, and not to the epimatium) represent a cup-like system pendent from the distal region of the ascending system.

It is of some interest to find a little confirmation of this view in the adjoining figure of *P. bracteata*, BLUME* (text-fig. 39). The section passed through a level about midway between the micropyle and the equator. It will be noticed that the ring of descending strands is incomplete on the "stalk" side, and, what is more important, that the line bridging the gap leaves the row of ascending strands an appreciable distance outside the ring.† The disposition of the mucilage canals, too, which form a complete circle of their own apart from the canals of the ascending strands, is probably not without significance. At a higher level the ascending strands approach the gap and finally themselves occupy the gap. A section from a level between the chalaza and the equator therefore gives the misleading impression that all the strands in the ring belong to the same system. (See STILES's fig. 4, *e*, *f*, p. 471, for *P. macrophyllus*, which will do equally well for the closely allied *P. bracteata*.)



TEXT-FIG. 39.—*Podocarpus bracteata*, BLUME. Transverse section of seed at a level midway between the micropyle and the equator. The vascular bundles are shown solid black.

* Synonymous with *P. neriifolius*, DON.

† I have observed the same feature in one other (unidentified) species of *Podocarpus* (§ *Eupodocarpus*), and consider it very probable that it occurs in several other species. The fact that STILES does not figure or describe the phenomenon in *P. macrophyllus*, a species closely allied to *P. bracteata*, may be due to his sections not having passed through the region to which this condition appears to be limited.

The conception of a cup-like vascular system pendent from the chalaza occurred to FAVRE (1865, p. 381) as early as 1865 from his study of *Podocarpus chinensis*, but, so far as I am aware, no one has followed up his suggestive idea.

In homologising the vascular system of the female organs of *Podocarpus* with that in *Acmopyle*, it must be recognised that what in *Podocarpus* is known as the "ovular stalk," adnate to the whole length of the seed and forming a distinct bulge in the transverse section, is in *Acmopyle* flattened out into a thin, curved lamina, so completely fused to the basi-dorsal surface of the seed that except for the curved ridge indicating its margin it would be impossible to detect its presence. The ascending strands of *Podocarpus* would correspond not only to the pair of large inverted basal strands in *Acmopyle*, here of very short length, but also to a portion of the dorsal valve of the cupule into which they distally expand. The distal limit of the "ascending system" of *Acmopyle* would probably be marked by the reflexed strands in text-fig. 26 which, as stated on p. 276, lie just under the ridge. That portion of the dorsal valve of the cupule which lies beyond the ridge would thus correspond to the "descending system" of *Podocarpus*. The ventral part of the cupule of *Acmopyle* must be regarded as a new acquisition, related in origin to the semi-erect position of the seed.

Although in broad outline, the above suggestion seems to afford a reasonable interpretation of *Acmopyle* in terms of *Podocarpus*, there remains one difficulty, which must at present remain unexplained, namely, the double nature of the cupule on the ventral side of the seed. Is it that the erection of the seed from the *Podocarpus*-like condition took place in two distinct stages, so that first the outer ventral arc was acquired and then the inner? The fact that of the two arcs the inner extends to a point nearer the chalaza (see text-fig. 6) than does the outer at any rate does not contradict this hypothesis. In this connection an examination of younger material would be of some interest. On the other hand, it may of course be that the process of erection is not merely ontogenetic, but is spread over the phylogeny of *Acmopyle*. In that case a *Podocarpus*-like form must have existed with a horizontal seed intermediate between these two genera.

We shall now consider STILES's theory that the elaborate vascular system of the seed of *Podocarpus* is not homologous with that in the older Gymnosperms. As stated on a previous page, I prefer to draw the line between the integument and epimatium just outside the ring of descending strands, considering only the peripheral portion of the flesh as the epimatium, and the inner region (containing the descending strands) as an integral portion of the integument. STILES, on the other hand, draws the line at the outer limit of the stony layer (1912, p. 475, and figs. 6, 7), thus assigning the descending as well as the ascending strands to the epimatium, and implying the absence of a sarcotesta proper to the integument. If the broken line in the figures cited were, indeed, the limit between the integument and epimatium, it is clear that STILES's theory would follow as a corollary, for the

epimatium, being homologous with the ovuliferous scale, is not an integral part of the ovule; its vascular supply cannot therefore be homologised with that of the Cycads, which is strictly ovular.

STILES, however, arrived at his conclusions by a different route. Arguing that the vascular system of the *Podocarpus* ovule is an elaboration of the rudimentary system in the more primitive members of the family, he concludes that the ovular system of the Podocarpeæ has been evolved within the family, and is therefore not homologous with that in the older Gymnosperms.

But I consider it possible that, during its evolutionary history, an organ may become much reduced, and subsequently attain a considerable development along different lines. Thus I regard the vascular system of the ovule of *Acmopyle* as homologous with that in the Cordaitalean ancestor, although in the modern genus the outer set of bundles has become well developed, while the inner has disappeared.

Unfortunately, the question is one that does not admit of a solution by positive histological evidence of any value. So far as I know, the descending strands have not been traced into the epimatium beyond its point of separation from the integument; on the other hand, it is equally true that they have not been traced into the free micropylar region of the integument.*

In the absence of direct evidence, therefore, we are left to whatever indirect evidence may be available from comparisons with other Gymnosperms. Perhaps the strongest argument against the theory advanced by STILES lies in the fact that the seeds of the Podocarpineæ, and, in fact, of all the Conifers, conform to the fundamental plan of structure of the typical platyspermic seed, and that, for this reason, such an important difference as that involved in the theory would be a *primâ facie* improbability.

Thus, two basal main supply strands, situated in the right-left plane, are an invariable rule throughout the Podocarps, as they are in the Cordaitales, *Ginkgo*, and the Taxineæ. When there is an inner or an outer system of strands (or when both systems are present), the strands, if unbranched, always lie in the plane of the carinæ (right-left plane), in the Cordaitales, *Ginkgo*, the Taxineæ, and in the only Pinaceæ in which they have been recorded, viz., *Abies* and *Cedrus* (RADAIS, 1894, Plates 2 and 6). In those species of *Podocarpus* in which there are only two descending strands, *e.g.*, *P. ferrugineus* and the species commonly known as *Dacrydium Bidwillii*, these always lie in the plane of the carinæ, and in *P. ferrugineus* they are actually in contact with the latter (SINNOTT, 1913, text-fig. 3, p. 46). In *Acmopyle*, too, it is significant that it is the carinal portions of the vascular cupule that reach nearest the micropyle.

* Miss GIBBS (1912) has given very useful figures of longitudinal sections of several species of *Podocarpus*, but from their diagrammatic character it is difficult to be sure of the correct positions of the strands. In any case they lie very near the limit. This author does not go into the question under discussion.

The correspondence, in the vascular organisation of the seed, between the Podocarpineæ and the remaining Gymnosperms, is so close that, unless there are strong reasons to the contrary, it is natural to recognise the homology of the different parts of the vascular system. It may therefore be concluded that the inner region of the fleshy seed-coat, which contains the "descending" strands, is really a part of the integument proper.

As regards the inter-relationships of the remaining genera of the Podocarpineæ, this subject has been admirably dealt with by STILES (1912, pp. 489–496), the main trend of whose argument appears to be convincing. I would only like to suggest that the genus *Dacrydium*, instead of forming a side line from *Microcachrys* (see the scheme on p. 496 of STILES's paper), may probably lie in a direct line, leading to *Podocarpus*, *Microcachrys* itself lying at the base of *Dacrydium*, as already indicated by STILES. It is rather surprising that, in the scheme proposed by STILES, *Dacrydium* does not appear as a link between *Microcachrys* and *Podocarpus*, for, from *Dacrydium Bidwillii* (as the plant was then known) to *Podocarpus*, the transition is so much more gradual than it is from *Saxegothæa* to *Podocarpus*.

Further, although I agree with STILES that *Saxegothæa* and *Pherosphæra* are two of the most primitive Podocarpineæ, I would not commit myself to any definite view as to their connections with the other genera of the family, especially as our knowledge of *Pherosphæra* is exceedingly meagre. The position of *Saxegothæa* is so clearly intermediate between the Podocarps and Araucarians (STILES, NORÉN, TISON, THOMSON) that it raises the question as to whether it would not be advisable to place this genus in a separate tribe, *Saxegothææ*, linking together the other two. It is significant that TISON (1909, p. 156) proposed the inclusion of *Saxegothæa* in the phylum Araucariales instituted by Prof. SEWARD and Miss FORD (1906, p. 398).

Phyllocladus appears to represent a branch leaving the main stem at about the level of *Dacrydium*, to which, apart from its specialised vegetative characters, it appears to show more resemblance than to any other Podocarp. The symmetrical nature of the epimatium would thus appear to have been secondarily acquired, for the ovule is erect throughout its development. Mrs. ARBER has already suggested that there is a correlation between the symmetry of the epimatium and the posture of the ovule.

B. *The Systematic Position of the Taxineæ.*

We may enter now upon the wider question of the affinities of the Podocarpineæ to the other groups of Conifers and the ancestry of the Conifers themselves. Since the discussion is intimately bound up with the inevitable question of the morphology of the ovuliferous scale, it is advantageous to make the latter a starting point.

But before dealing with this subject it is necessary to point out that I have ventured to exclude from the phylum Coniferales the genera *Taxus*, *Cephalotaxus*, and *Torreya*. Both the general morphology and the internal structure of the fructifications lead me to the conclusion that the family Taxineæ is too distinct to occupy

a place among the Conifers, and that it should rank as an independent phylum, Taxales,* related to *Ginkgo*, to the Cordaitales, and to the Coniferales as here defined.

In LINDLEY'S 'Vegetable Kingdom' (1853, p. 231), the Order Taxaceæ, which included the Yews and Podocarps† as well as *Ginkgo*, appeared as a group of equal rank with the Cycadaceæ, Gnetaceæ, and Piniaceæ. The latter group was also named Coniferæ, a term which has since acquired a wider significance, for it now covers both the Pinaceæ and Taxaceæ of LINDLEY, with the exception of *Ginkgo*.

In the most up-to-date general work on Gymnosperms (COULTER and CHAMBERLAIN, 1917) these two groups appear as equivalent orders of the phylum Coniferales. However, as Dr. SCOTT (1911, p. 172) has remarked, "it is doubtful whether even this first grouping is natural, for the Podocarps among the Taxaceæ seem to have little to do with the Yews."

The opinion that the relation between the Yews and the Podocarps is not a close one dates at least as far back as 1838, when BENNETT and BROWN (1838, p. 36) wrote as follows: "It is singular that so unessential a character as the general solitary position of the ovuliferous scale should have induced all the authors who have attempted an arrangement of the Coniferæ to separate *Podocarpus* and *Dacrydium* from the true Pines and to associate them with the Yew." PILGER expressed exactly the same view in 1903 (pp. 25, 38). In 1910, BROOKS and STILES (p. 316) observed: "The points of similarity between the Podocarpeæ and the Taxeæ do not seem to us to be very striking;" and in 1912, STILES (498–499) wrote: "It is at least safe to say that there is as yet little evidence of any near connection of the Taxeæ and Podocarpeæ," at the same time enumerating several important structural features in which the two groups show a "marked contrast."

Remembering that LINDLEY was unaware of the presence of motile sperms in *Ginkgo*, his placing that genus in relation to *Taxus*, *Cephalotaxus*, and *Torreya* may be regarded as a natural step; but in including *Dacrydium* and *Podocarpus* in the same Order as *Taxus*, he was probably unduly impressed by the tendency in all these genera towards a fleshy development of some parts of the female fructification, which may not necessarily be homologous. The Yew berries, for example, closely resemble the dry seed of *Dacrydium laxifolium* perched upon the delicate fleshy receptacle composed of swollen bract bases.

In 1906, Mrs. AGNES ARBER (Miss ROBERTSON, 1906, p. 101), expressed the opinion that "the general morphology of the female 'flower' of *Taxus* more closely recalls that of *Cordaites* than that of any other known plant." At the same time *Ginkgo*, itself showing strong Cordaitean features, has long been known as an ally of *Cephalotaxus*—an alliance which, as Dr. SCOTT observes (1906, p. 143), was

* A name previously employed by LOTSY (1911, p. 160), but not in the sense of a group equal in rank with the Coniferales.

† With the exception of *Pherosphæra*, *Saxegothæa*, and *Microcachrys*. In his 'Natural System' too (1836, p. 317) LINDLEY had associated *Taxus* with the genera *Podocarpus*, *Dacrydium*, *Phyllocladus*, and *Ginkgo*.

established before the discovery of the motile sperms. If we agree with Dr. SCOTT that the Yews have little to do with the Podocarps, it may confidently be added that they have still less to do with any other Conifers.

Had it not been for the genus *Phyllocladus* which chiefly on account of its symmetrical epimatium, appears to link the remaining Podocarps with the genus *Taxus*, it is extremely probable that the Order Taxaceæ would not so long have preserved its integrity. It is, therefore, of importance critically to examine the evidence upon which *Phyllocladus* claims a Taxinean affinity.*

It is noteworthy that with the exception of PILGER, who placed *Phyllocladus* in a tribe intermediate between the Taxineæ and Podocarpineæ, nearly all the modern writers on the subject have definitely expressed the view that the affinities of the genus are clearly with the Podocarps, and have considered it unnecessary to retain the intermediate tribe (KILDAHL, 1908, p. 465; YOUNG, 1910, p. 91; BROOKS and STILES, 1910, p. 316; STILES, 1911, p. 346; STILES, 1912, p. 445).

Mrs. AGNES ARBER (Miss ROBERTSON, 1906)—who was the first to investigate this interesting genus in detail—while fundamentally agreeing with this view, appears to have been impressed, rather more than the other authors, by certain features in which *Phyllocladus* seemed to approach the Taxineæ rather than the Podocarpineæ. However, at the time when Mrs. ARBER wrote her lucid paper on *Phyllocladus* our knowledge of the Podocarpineæ was very meagre: in the light of facts which have since been observed, out of the few characters which were then suggested as being Taxinean rather than Podocarpean, all except one appear to lose much of their value.

(i) *Presence of Centripetal Wood in the Cladodes.*—Mrs. ARBER herself does not lay much stress on this point, and I think justly. It is still a moot point whether transfusion tracheides found in the vicinity of the protoxylem, and often separated from it by parenchyma, should be reckoned as true centripetal xylem. But whatever view be adopted on this point, the tissue referred to in *Phyllocladus* is found in numerous other Conifers (including *Dacrydium*, *Podocarpus*, *Acmopyle*) in exactly the same relative position, and without leaving any doubt as to its identity with it.

Although in *Phyllocladus alpinus* Mrs. ARBER records the presence of this tissue only in the lateral bundles of the cladode, in my preparations of *P. asplenifolius* and *P. trichomanoides* (the latter closely allied to *P. alpinus*) it occurs not only in all the strands of the branch-system of the cladode, but also in the scale-like leaves of the unmodified shoot.

* In all the following features it differs from the Taxineæ, while at the same time resembling the Podocarps:—The form and structure of the male cones and stamens; winged pollen; male gametophyte; the general morphology of the female cone with each ovule always clearly subtended by a bract; free nucellus (entirely absent in the Taxineæ, but present in some Podocarps); presence of spongy tissue and a thick two-layered megaspore-membrane (a feature of doubtful value; although conspicuously absent in the Taxineæ it is also absent in some Podocarps); general vegetative anatomy, such as the presence of root tubercules and entire absence of bar thickenings in the secondary wood; and, finally, the southern distribution of the genus (the Taxineæ being chiefly northern forms, the Podocarpineæ chiefly southern).

(ii) *Taxinean Sculpturing.*—Mrs. ARBER discovered that the tissue just referred to, for which I prefer the non-committal name of transfusion tracheides, showed in *Phyllocladus* a peculiar type of sculpturing, which combined small scattered narrowly bordered pits with band-shaped thickenings; the latter passed round the cells in various directions, specially when the cells were iso-diametric. A rather similar type of ornamentation was recorded in the "normal" xylem. By the latter term I understand that only the primary xylem is meant; for in the two species examined by me (one of which, as already stated, is very similar to *P. alpinus*) I could not discover any trace of band-like thickenings in the secondary wood, and it would be surprising if these were found in *P. alpinus.**

It is doubtful whether we can justly refer to this feature as true Taxinean sculpturing, which is rather different in appearance and *is always present in the secondary wood.* Moreover, it is important to mention that I have observed exactly the same type of tracheides as those recorded in *Phyllocladus*, in the primary xylem of *Acmopyle* (see text-fig. 1), in two species of *Podocarpus* and in *Dacrydium laxifolium.* In none of the members of this family, however, does the secondary wood show any band-like thickenings at all.

(iii) *The Erect Posture of the Seed.*—As Mrs. ARBER herself states, a nearly erect seed is also found in *Dacrydium laxifolium*† and, we may add, on the authority of PILGER, in *D. Fonkii*, *D. Franklinii* and, of course, *Acmopyle.* In some ripe material of *D. laxifolium* and *D. cupressinum* examined by me the seeds are perfectly erect; the same condition is figured by KIRK in *D. intermedium* (1889, Plate 87B), and also in the two former species.

However, the seed is in these cases not erect in the same sense as in *Taxus* where the nucellus is evidently from the very outset the direct continuation of the seminiferous axis. The condition in *Taxus* is more readily compared with *Cordaites* and *Ginkgo* than with any of the Podocarps.

(iv) *The Symmetrical Epimatium.*—It cannot be denied that this character is more Taxinean than Podocarpean, but there appears to be some reason for the view that the symmetry of the epimatium of *Phyllocladus* has been secondarily acquired (see above, p. 287). This feature, as suggested by Mrs. ARBER, is probably correlated with the erect position of the seed, exception being made, of course, in those cases where an inclined ovule with an asymmetrical epimatium subsequently becomes erect, as in *Dacrydium.* In *Acmopyle*, too, the epimatium is symmetrical, at least in the major portion of the seed—a fact which, by the way, would seem to suggest that the young stage of the ovule is probably not much more inclined than the ripe seed, or at least not completely inverted.

* Since the above was written I have been able to confirm their absence in this species from Mrs. ARBER'S original preparations, which she most generously placed at my disposal for comparison.

† The spelling of the specific name as "latifolium," repeated by Miss KILDAHL (1908, p. 465) is evidently a misprint.

Mrs. ARBER justly observed that it would not do to lay too much stress upon the usually asymmetric nature of the epimatium of the Podocarpeæ, as showing that this organ is not homologous with the aril of *Taxus* and *Torreya*. At the same time, the symmetrical epimatium of *Phyllocladus* and *Acmopyle* does not necessarily indicate a homology with the aril of *Taxus*, for any extra seed-coat would tend to develop symmetrically if subjected to uniform surroundings, such as obtain in the case of erect ovules. It would seem, therefore, that the symmetry of the epimatium of *Phyllocladus* speaks neither definitely for nor against its homology with the aril of *Taxus*.

The Taxales stand apart from the Coniferales in the general organisation of their ovuliferous shoots and in the retention of obviously primitive characters in the seed (OLIVER and CHICK, 1902; OLIVER, 1902) and seedling (CHICK, 1903). They are the only living Gymnosperms which, having relinquished the zoidiogamic mode of fertilisation, still linger behind the others in preserving the vanishing traces of an internal vascular system in the seed (OLIVER, 1903). The male cones are very distinct from those of the Conifers, especially in the habit and in the frequent presence of bracts between the groups of stamens. The importance of the presence of bracts (*cf.* Cordaitales) is probably very great. The individual stamen, too, is in *Taxus* totally unlike that in any Conifer, although in *Torreya* there are unmistakable signs of progress in that direction by a gradual departure from the peltate form. In spite of this, however, the mode of insertion and general appearance of the branched bracteate male cone of *Cephalotaxus* remains strikingly different from that of any known Conifer.

The entire absence of vegetative prothallial cells in the male gametophyte may also be reckoned as a feature of some importance in distinguishing the Taxales, for although it is found also in the Taxodineæ and Cupressineæ, these two tribes are in most other respects so markedly different from the Yews that this resemblance can hardly be traced to a near relationship. A similar argument applies to the so-called "taxinean sculpturing" in the secondary wood, for it occurs in some of the Abietineæ (*Pseudotsuga*, *Larix*, *Picea*; see GOTHAN, 1905, p. 98)—a tribe of Conifers which probably has very little to do with the Taxales.

It is difficult to say what significance can be attached to ROTHERT'S discovery of so-called "parenchymatous tracheides" in the pith of a species (or perhaps only a variety) of *Cephalotaxus* (1899, p. 275). From ROTHERT'S account and from some preparations which Mrs. ARBER very kindly allowed me to examine, these tracheides do not essentially differ from the familiar transfusion tracheides associated with the leaf-strand in numerous Conifers. In their presence in an axial organ they are certainly unusual, but not unique, for similar elements occur also in the branch system of the cladode of *Phyllocladus* (see p. 289). Mention may also be made of the fact that the presence of secretory canals in the pith of the stem—a very uncommon feature in which the genera *Ginkgo* and *Cephalotaxus* agree—has been emphasised by VAN TIEGHEM (1891A, p. 189), as a sign of affinity.

The position adopted by several writers, such as R. B. THOMSON (1905, pp. 45 and 54), LAWSON (1907, p. 20), COULTER and CHAMBERLAIN (1917, p. 418), and BLISS (1918, p. 56), that the Yews represent a relatively modern group, appears to be untenable in view of the evidence from the morphology of the male and female organs, specially the presence of bracts in the male cone and the structure of the seed.

The absence of a thick megaspore membrane has been employed by Prof. THOMSON (1905) in support of the view that the Taxineæ are a recent group. This author examined *Taxus canadensis* and states (p. 45): "The later stages of *Taxus* up to maturity of the seed gave no indication of the presence of a megaspore-coat." However, in *T. baccata* a species closely allied to, if not identical with *T. canadensis*, I found that, although the megaspore membrane is really a "negligible feature" at the early free-nuclear stage of the prothallus, at the time of embryo formation it is of appreciable thickness. While the thick membrane of the Cycads and *Ginkgo* is thoroughly in accord with their primitive nature, the value of this criterion in the other groups is still doubtful, specially in the Podocarpineæ, among which both thick and thin megaspore membranes occur. In particular I may mention that in my preparations this variation is even seen within the genus *Podocarpus*, which, so far as I am aware, has hitherto been known only to have a poorly developed membrane, in contrast to *Dacrydium*. Thus the two species of *Podocarpus* hitherto known as *Dacrydium Bidwillii* and *D. Kirkii* (see p. 282, footnote*), as well as *Podocarpus alpinus* (§ *Eupodocarpus*) have each a well-developed megaspore membrane like that of *Dacrydium* or *Phyllocladus* (ROBERTSON, 1906). One other species belonging to § *Eupodocarpus* shows a medium thickness.

Prof. OLIVER'S work has conclusively shown that *Torreya* possesses one of the most primitively organised seeds among living Gymnosperms. From his study of the seed of *Cephalotaxus*, WORSDELL (1900, pp. 317–18) has come to a similar conclusion with regard to this genus. The resemblance in the general morphology of the female shoot of *Taxus* and *Cordaites* has also been widely recognised. These considerations impelled me to search for possible archaic features in the mature seed of *Taxus*, and to attempt to describe it in terms of *Torreya*, which, according to Prof. OLIVER'S well-known theory, is not difficult to derive from a type like *Cardiocarpus*. The result of this search has been to convince me that the seed of *Taxus* is in some respects even more primitive than that of *Torreya*, appearing, in fact, to form the terminal link in a remarkable chain of seed-types, leading from *Cardiocarpus* through *Cycadinocarpus*, *Rhabdospermum*, *Mitrospermum*, and *Taxospermum*, to the living genus *Taxus*.

It would take us too far here to discuss the relations between the different stages of this series, which, although not by any means suggested as representing the actual course of evolution, yet appears to illustrate progression along certain well-marked tendencies, which would seem to bridge up the structural gap between the seeds of

the Cordaitales and those of the Taxales. I hope shortly to deal with this subject in a separate paper.*

C. *The Ovuliferous Scale of Conifers.*

In the entire domain of plant morphology, there is no structure which has exercised so wide an influence upon our views on the phylogeny of a group as the ovuliferous scale of Conifers. Out of the mass of literature, dating from nearly a hundred years ago, two rival theories stand out prominently: (1) the Brachyblast Theory; (2) the Ligular Theory.

The question is so wide, and has so often been handled by experts, that I cannot pretend to deal with the entire evidence. I shall, however, venture to go into some detail with regard to the evidence from the vascular anatomy, which, I may say beforehand, appears to me to be conclusively in favour of the Brachyblast Theory.

(a) *Evidence from Vascular Anatomy.*—The most striking fact that emerges from a general survey of the literature† is that, at its origin, the supply to the ovuliferous scale is fundamentally similar to that to a vegetative axillary branch, while sometimes the resemblance is so close that a distinction appears impossible. The similarity between the vascular supplies of the ovuliferous scale and vegetative axillary bud was frequently urged by STRASBURGER in his classical work on the Conifers and Gnetaceæ (1872, frequent statements scattered over pp. 25–75, in connection with *Biota*, *Juniperus*, *Araucaria*, *Agathis*). More recently, EAMES (1913, p. 21) has laid emphasis on the same feature. But no author has illustrated this point so clearly as Miss AASE (1915) has quite recently done in a detailed paper on the vascular anatomy of the megastrobili of Conifers. A comparison of her figures, specially of *Pinus maritima* and *P. Banksiana* (pp. 286, 287), besides numerous others, is alone sufficient to demonstrate the identity of the vascular relations; in both the ovuliferous scale and the vegetative axillary bud the supply comes off typically from the sides of the foliar gap in the axial cylinder.

The vascular structure of *Acmopyle*, too, shows the fundamental similarity between the supply to the ovuliferous scale (or its vestige) and the vegetative axillary shoot (see above, pp. 267, 268, and text-figs. 5, 7–18). In this connection, a comparison of text-figs. 5 and 10 of the present paper with Mrs. ARBER'S (Miss ROBERTSON'S) figure of the vegetative stem of *Phyllocladus* (ROBERTSON, 1906, Plate 17, fig. 7) and STRASBURGER'S figure of *Sciadopitys* (1872, Plate 26, fig. 5) is particularly illuminating.

From such data as these, the axial nature of the ovuliferous scale appears to be clearly demonstrated.

Soon after its origin, the supply to the scale becomes arranged in the form of an

* This paper has, since the above was written, been published in the 'Annals of Botany,' vol. 34, pp. 117–133, January, 1920.

† See especially the extensive researches of VAN TIEGHEM (1869), STRASBURGER (1872), RADAIS (1894), AASE (1915).

arc, having an orientation inverse to that of the subtending bract-trace.* This inverse orientation of the vascular supply, while being intelligible on the brachyblast view, is an obstacle in the way of the ligular theory. EICHLER (1881, p. 1026) states that the inverted orientation is a property of the supply to all excrescences of the leaf, whether they come off from the upper or from the lower face, but it is doubtful if this statement is at all of general application. It fails, as EAMES has already pointed out, in the irregular orientation (most often concentric) of the ovular strands in many of the Conifers studied by RADAIS (1894, p. 286; see especially his figures of *Abies*, *Tsuga*, and *Sciadopitys*). Miss AASE (1915, p. 287) also records concentric ovular supply strands in *Keteleeria Fortunei*. Since EAMES has already dealt with THOMSON'S (1909) statement relating to the alleged two inversions (one for the ovuliferous scale and one for the ovules), I will here only express my complete agreement with the former author.

As already noticed, in the majority of the cases on record, the ovuliferous scale supply showed a striking agreement with the supply to a vegetative axillary bud; it had an independent origin from the sides of the gap in the cone-axis cylinder. In other (comparatively few) instances the ovuliferous scale supply was described as branching off from the bract supply, and these instances have been employed in support of the ligular theory. Until recently, except for one or two genera, such as *Araucaria* and *Microcachrys*, there were no cases on record in which both these modes of origin were known in the same genus, nor was it generally realised that the relative sizes of the two parts of the cone-scale are subject to considerable variation, even in different regions of the same cone.

Thus THOMSON (1909) was led to believe that while in the Abietineæ (and, by inference, in the Taxodineæ and Cupressineæ) the cone-scale was evidently a double structure and the ovuliferous organ a modified axillary shoot, in the Araucarineæ and Taxaceæ (incl. Taxineæ and Podocarpineæ) the scale was simple, and the ligule of *Araucaria* and the epimatium of *Podocarpus* were mere excrescences of the upper surface. He thus founded two great groups of Conifers, the Diplosporophyllous and the Aplosporophyllous forms. This step has appropriately been criticised by EAMES (1913, p. 33).

More recently, Miss AASE (1915) has published facts which are of value in this connection, showing that the classification proposed by THOMSON is not tenable. A glance at her fig. 1, p. 283, will show that, according to THOMSON'S classification, the scales in the proximal region of the cone are aplosporophyllous, while those higher up in the same cone are diplosporophyllous. In *Pinus maritima*, *P. Banksiana*, *Keteleeria fortunei*, *Cupressus Benthamii* Miss AASE describes both types of vascular

* The vascular cylinder of the brachyblast is to be conceived of as having a gap opposite to the subtending bract, as is usually the case with the vascular cylinder of the vegetative axillary shoot in its basal region, not only in the Conifers but also in many Ferns. If the ovuliferous scale is really a branch, it is evident that, strictly speaking, there is no sense in describing, as inverted, the strands on the side opposite to that on which the bract is placed.

supply (independent and joint) in different parts of the same cone. After reading her paper, I found the same feature in *Pinus silvestris*; I have also described it above in *Acmopyle* ('Vascular Anatomy of Receptacle,' p. 265 *ff*).

It is interesting to remark that almost all the different vascular relations between the ovuliferous scale and bract of Conifers are paralleled among the Ferns by those of the axillary branch and its subtending leaf; the analogy is indeed so close that the condition in the Ferns may throw light upon the morphology of the ovuliferous scale in Conifers. Those cases among Conifers in which the scale supply appears to originate partly or entirely in the trace of the subtending bract are analogous to the condition in the Hymenophyllaceæ and numerous other Ferns, in which the branch (whether adaxial or abaxial to the associated leaf) may be adnate to the petiolar base for a variable distance. In such cases it is entirely out of the question to regard the branch as an outgrowth of the leaf. As pointed out elsewhere (SAHNI, 1917, p. 19), the basal undivided portion of the vascular supply must be regarded as having a dual nature, partly axial, partly foliar. STRASBURGER (1872, p. 69) has also drawn attention to somewhat similar analogies among Angiosperms, of which the adnate inflorescence of *Tilia* affords the most familiar instance.

The fact that the supply to the ovuliferous scale appears to arise from the supply to the bract, therefore, does not necessarily indicate that the one organ is morphologically an excrescence of the other.

(b) *Degree of Development of the Ovuliferous Scale as an Index of Affinities.*—Considerable importance has been attached by several authors to the degree of development of the ovuliferous scale as an index of affinity or of relative antiquity. While the exponents of the ligular theory have naturally interpreted the poorly developed ovuliferous scale of *Araucaria* and *Agathis* as signifying primitiveness, Prof. JEFFREY and his pupils, who believe in the brachyblast theory and in the Abietineous origin of the Conifers, have made use of the fact that in *Pinus* and allied genera the ovuliferous scale (in the middle region of the cone) appears more nearly to resemble an axillary shoot than it does in *Araucaria*. Finally, against those who advocate a Cordaitean ancestry for the Conifers, the criticism has been made (SEWARD and FORD, 1906, p. 393) that as we pass backwards in geological time we do not find an increasing development of the ovuliferous scale, which would bring the older Conifers (Araucarineæ) more nearly into line with *Cordaites*.

We have seen, however, that the degree of development of the ovuliferous scale is variable, not only within narrow circles of affinity (EAMES, 1913, on *Athrotaxis* spp.), but even in different regions of the same cone (AASE, 1915). Keeping in view the analogy with the Ferns, where, as we know, the relative development of the branch and its associated leaf does not bear any definite relation to the systematic position of the plant, one is naturally inclined to ask whether even among the Conifers too much importance has not been attached to the degree of development of the ovuliferous scale as an index of relative primitiveness.

D. *The Ancestry of the Conifers.*

(a) *Relative Antiquity of Abietineæ and Araucarineæ.*—If we exclude the Taxineæ, as suggested in this paper, there remain two groups, the Abietineæ and the Araucarineæ which, respectively, according to two rival schools of thought, claim to be the oldest living Conifers. It would be superfluous to repeat the evidence adduced by the two sides—both claim the support of the fossil record as well as that of the structure of living plants, and the controversy is certainly a beautiful example of the way in which practically the same data can be construed in diametrically opposite directions.

While the palæontological evidence (SEWARD and FORD, 1906) places beyond doubt the existence of Araucarians in the Palæozoic, the existence of Carboniferous and Permian Abietineæ has been shown to be without any proof. Of two fossil woods supposed by JEFFREY and CHRYSLER (1906) to belong to Palæozoic Abieteneæ, one has been shown to be not Abietineous and the other is equally certainly not Palæozoic. THOMSON and ALLIN (1912) have clearly demonstrated that the supposed horizontal resin-canals of the wood named by PENHALLOW *Pityoxylon chasense* (from the Permian of Kansas) are really the persistent leaf-traces of a *Dadoxylon* devoid of annual rings. The other wood, *Pinites Conwentzianus*, Goepp., the Abietineous nature of which Prof. SEWARD has confirmed by an examination of the original sections, was believed by GOEPPERT to be of Carboniferous age. The fossil was, however, found on a rubbish-heap, so that its correct age is extremely doubtful, and Prof. SEWARD has recently obtained further confirmation of this doubt (SEWARD, 1919, p. 220).

Both from the structural and from the historical side, the balance of evidence is at present decidedly in favour of the Araucarineæ being the more primitive group, and the resemblances which the latter group shows to the Cordaitales (BURLINGAME, 1915) are in accord with the view that they are more primitive than the Abietineæ. However, the facts at our disposal do not appear definitely to indicate that the one group is necessarily derived from the other. An independent origin from a common source is, at any rate, a safe hypothesis.

(b) *Fossil Podocarpineæ.*—Unfortunately, the fossil history of the Podocarpineæ—the family with which we are here more intimately concerned—is very imperfectly known. This is probably because, as BURLINGAME has hinted (1908, p. 175), "we know but little of the plant-remains of those parts of the world in which their remains would be most likely to be found." The probability of a relation with the Araucarians justifies the hope that undoubted Podocarpineæ may be discovered in the Mesozoic rocks, but it is for the future to show whether this group can at all rival in age the Araucarineæ.

Apart from abundant petrified wood of the *Podocarpus* type, numerous impressions have provisionally been referred to the Podocarpineæ.* Of these impressions, one

* The literature has been well summarised by STILES (1912, pp. 486–89); a more complete account is given in the fourth volume of Prof. SEWARD'S 'Fossil Plants.'

of the oldest, and also the most interesting, is the Rhaetic genus *Stachyotaxus*, NATHORST (1908, p. 11, Plates 2, 3), whose correct systematic position it is of great importance to ascertain, for it may, as hinted by Prof. NATHORST (p. 15, footnote), form a link between the Cycadophyta and the Conifers. The resemblance of this genus to *Cephalotaxus* is perhaps, on the whole, more striking than that to *Dacrydium*, in view of the paired ovules, symmetrical cup at the base, and the biseriately arranged leaves. But, as Prof. NATHORST says (*loc. cit.*, p. 15), the habit of the vegetative shoot is strikingly suggestive of *Podocarpus imbricatus*.*

The large two-winged pollen-grains discovered by Prof. NATHORST (1908, Plate 2, figs. 53–55) in the Triassic rocks of Sweden, and compared with pine-pollen, may equally well belong to extinct Podocarpineæ, for we are at present without any knowledge as to the contents.

A passing reference was made on a preceding page to a faint resemblance between the sculpturing of the sclerotesta of *Acmopyle* and that of the Palæozoic seed *Stephanospermum caryoides*, Oliver. Although this can only be regarded as a distant analogy, it was considered worth while to give detailed figures of the stone of *Acmopyle*, in view of the possibility that seeds with a similar pattern may be discovered in a fossil condition from the southern hemisphere.

It is also difficult to say what justification there may be for homologising with the "tent-pole" of *Ginkgo* and *Cordaites* the cone-like projection at the apex of the female prothallus of *Acmopyle* (see text-fig. 6). In order to see how far this feature is repeated in other members of the family, a search was made of the few published figures, showing the apex of the female gametophyte. Miss GIBBS (1912, Plate 49, fig. 6) figures a distinctly pointed apex in *Podocarpus imbricatus*. Miss YOUNG'S figure of *Phyllocladus* (1910, Plate 6, fig. 36) shows a condition particularly suggestive of *Ginkgo* and *Cordaites*, for there is a distinct central projection, surrounded by what looks like a deep trough, into which the archegonia open, but, in the absence of figures of transverse sections, I am unable to say if the archegonial depressions pass completely round the central peg.

(c) *Ultimate Origin of Conifers as a Whole.*—Whether the Conifers arose ultimately from megaphyllous (Pteropsid) or microphyllous (Lycopsid) ancestors is a question which cannot yet be regarded as settled, although it has engaged the attention of a number of eminent botanists for many years.† The more closely one tries to examine the evidence, the more distant does the solution appear to become, and I find it impossible to express a definite opinion. It goes without saying, that our knowledge of the older vegetations of the world is yet far from satisfactory ; in the Devonian, or, it may be, in even older rocks, probably still lies buried the

* The figures (NATHORST, 1908, plate 2, figs. 19–24) also make it probable that *Stachyotaxus elegans* at least shares with *P. imbricatus*, *Acmopyle*, and *Dacrydium falciforme*, etc., the peculiar laterally compressed leaf-blade, for there appears to be no twist in the leaf-base.

† The discussion has recently been well summarised by BURLINGAME (1915).

answer to the question: Is the division of vascular plants into Pteropsida and Lycopsida so fundamental that it existed at their very origin from the non-vascular plants, or was the one class derived from the other? Naturally, if the megaphyllous forms have ultimately originated from microphyllous ones, the apparently divergent views as to the origin of the Conifers can to some extent be reconciled with each other. Thus, I believe it may be stated with confidence that the relatively near ancestors of the Conifers were forms like the Cordaitales. But the possibility of a remote Lycopsid connection,* without the intervention of true ferns or fern-like seed-plants, may not perhaps be entirely excluded, although at present there does not appear to be much evidence of a positive nature in support of such a connection.

As is well known, Dr. Scott (1909, pp. 650–652) has emphasised certain points of resemblance between the Cordaiteæ and the Pteridosperms, in support of the conclusion already expressed by him in 1902 (Scott, 1902) that the Cordaiteæ sprang from a Pteridosperm stock. In the first place, the seed in the two groups is constructed on the same general plan; secondly, by a gradual loss of centripetal xylem the stem anatomy of certain Pteridosperms passes into that of the typical Cordaiteæ. A number of Cordaitean stems are also known which approach the Pteridosperms in the possession of centripetal wood. In recent years the evidence for such an anatomical transition in the stem has been steadily increasing, and to express their intermediate structure, some of these Cordaitean stems were grouped by Scott and Maslen (1910) under the genus *Mesoxylon*, of which several species have now been described (Scott, 1912, 1918, 1919; Maslen, 1911).

So far as present knowledge goes, the Pteridosperms undoubtedly represent the nearest approach to the hypothetical ancestors of the Cordaitales. At the same time, there are important points of difference between the two groups, which have, of course, been recognised by Dr. Scott, but which may perhaps deserve as much emphasis as he has justly laid upon the resemblances. I wish to guard against the impression that I consider an origin of the Cordaitales from a Pteridosperm stock as improbable, but the wide differences between the two phyla, at any rate as we know them at present, do not readily fall in with this view.

* Naturally, an affinity so remote as may here be implied can hardly be expected to find expression in detailed structural resemblances: as may be inferred from my above-expressed belief in the brachyblast theory of the ovuliferous scale of Conifers, I do not agree with Schumann (1903, p. 71) in regarding the ligule of the Heterosporous Lycopods as being homologous with the organ so named in *Araucaria*. If such a homology existed, it might be reasonable to expect that the latter genus should preserve a trace of the ligule on the microsporophyll as well, if not on the vegetative leaf, in order to materialise the comparison with the Heterosporous Lycopodiales, which are never devoid of a ligule, even in the vegetative leaves. Schumann (1903, p. 70), who believes in the Lycopod origin of all Conifers, is so deeply impressed by the resemblances between the *Lepidocarpon* and the ovuliferous organ of the Conifers, that he considers it by no means certain that the Lepidodendreæ have correctly been assigned to the Pteridophyta. But the ligule of the Heterosporous Lycopods appears still to be a morphological enigma.

A review of the entire Gymnospermous series shows that there are two great divisions, based primarily upon the manner in which the seed is borne, whether upon the leaf or directly upon the axis. In the Pteridosperms and Cycads the seeds are always clearly borne upon the leaves, while in the Cordaitales (so far as known) and in the Ginkgoales, Taxales, and Coniferales they are borne either clearly upon an axis, or upon a structure which is probably some modification of an axis.* This differentiation of the Gymnosperms into two divisions, which I here propose to refer to as the Phyllosperms (with leaf-borne seeds) and Stachyosperms (with stem-borne seeds), respectively, appears to be very old, for, as far as I am aware, there is among the Pteridosperms no sign of a transition from the phyllospermic to the stachyospermic condition: a sharp differentiation is seen as far back as the available fossil records can take us. There could scarcely be a greater contrast in this respect than there is between RENAULT'S well-known figure of *Cordaianthus Williamsoni* (see SCOTT, 1909, p. 540) and the equally familiar figure of *Lyginopteris Oldhamia*, which forms the frontispiece of Dr. SCOTT'S 'Studies.'

Combined with this fact there are other important features, in which, as Dr. SCOTT points out (1909, p. 650), the Cordaiteæ and the fern-like seed-plants are as far apart as two groups of Gymnosperms very well can be. The strap-shaped parallel-veined leaves of *Cordaites*, and in fact the leaves of any known Stachyosperm, living or fossil, are entirely different from the much-divided fern-like fronds of the Pteridosperms or the almost equally complex leaves of the Cycads. The result is that in habit the two groups are totally distinct, and it is not easy to picture a Pteridosperm frond reduced into the simple Cordaitean leaf—at any rate, no intermediate form is yet known. Our knowledge of Cordaitean and Pteridosperm leaves is by no means complete, and transitional forms may yet be discovered; but until these are forthcoming it seems necessary to give due importance to the sharp contrast between the two types.

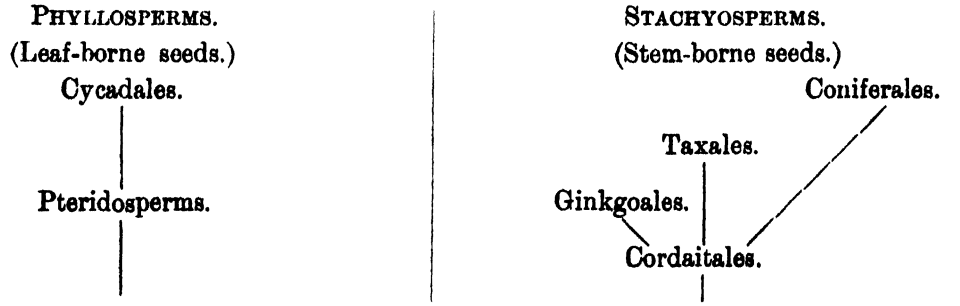
It is difficult to say whether we can extend to *Cordaites* the so-called phyllode theory of the leaf, originally applied to the monocotyledonous leaf. According to this theory, the typical monocotyledonous leaf represents only the proximal non-laminated region of the typical dicotyledonous leaf. Quite recently Mrs. AGNES ARBER (1918, p. 472) has published a paper of unusual interest, in which she brings forward convincing anatomical evidence which I think places the theory upon a firm basis, so far as the monocotyledons are concerned. In the leaves of *Cordaites* and the Conifers, however, similar anatomical evidence appears to be lacking. Nevertheless, I

* The importance of this difference between the Cyads and the Conifers was emphasised by STRASBURGER as long ago as 1879 (p. 139). As to the Gnetales and Bennettitales, further investigation is needed to show clearly whether they belong to the first category or to the second. STRASBURGER placed the Gnetales among the former class. On the other hand, in view of the far-reaching resemblances, in vegetative characters, of the Bennettitales with the Cycadales, the rather sharp contrast in the reproductive organs is disconcerting. The view has, however, been expressed (SEWARD, 1917, p. 382) that the seed-bearing pedicels of the Bennettitales are morphologically of a foliar nature.

consider it likely that the cupressoid type of leaf may have been produced by reduction from a taxoid leaf, and the latter from the multinerved type found in *Cordaites*, particularly as leaves similar to that of *Cordaites* also occur in the Araucarineæ. But the difficulty appears to lie in deriving the Cordaitean or Araucarian leaf from the much-divided fern-like frond, and this, it will be agreed, is the crux of the question. It is gratifying to learn that Mrs. ARBER hopes to return to this aspect of the phyllode theory, and her results may be awaited with interest, for light may be thrown upon the important question as to whether the apparent microphylly of the Conifers is due to reduction from a Pteridosperm-like condition or a reduction merely within the Conifers.

In the male organs, too, there is no clear resemblance between the Cordaitales and the Pteridosperms, the latter, as Dr. SCOTT points out, having no definite "cones." There appears to be hardly a comparison—except a rather distant one in form—between the "epaulets" of *Crossotheca* and the "stamens" of *Cordaites*. The one represents a minute portion of a dissected leaf; the other, according to the view which appears the most plausible, is itself an entire foliar organ, attached directly to the axis.* Each "epaulet" of *Crossotheca* may possibly be compared to a sorus on the microsporophyll of a Cycad, but not with the "stamen" of *Cordaites*; the latter organ is probably homologous with the microsporophyll of the Taxales, in which group the presence of interstitial sterile bracts in the male cone (*Cephalotaxus*) may considerably enhance the resemblance with *Cordaites*.

If the Stachyosperms and the Phyllosperms have had a common origin from megaphyllous ancestors it is clear that we must await the discovery of forms which show a transition from the leaf-borne to the stem-borne seed, as well as a reduction of the much-divided fern-like frond to the simple leaf invariably found in the Stachyosperms. A rather similar reduction must also be visible in the male organs. So long as these important links are missing there is at least some strong negative evidence against the megaphyllous theory.†



* If it is held, with SOLMS-LAUBACH, that the structure here called a stamen is a male flower (see SCOTT, 1909, p. 537), the above comparison would be still less valid.

† Since the above was written Dr. SCOTT (SCOTT, 1920) has read a paper bearing on the ultimate origin of the Pteridosperms, and expressed the view that they "have always been distinct from any of the known phyla of vascular Cryptogams . . . parallel in important respects to the ferns, but of unknown and remote origin." Unfortunately, my knowledge of this paper is still limited to the brief reference in 'Nature.'

Much has recently been accomplished in connecting up the Cordaiteæ with certain Pteridosperms by means of Cordaitean forms showing an intermediate stem-anatomy, but light has still to be thrown upon the nature of the fructifications borne by these plants. The most recent work on the subject (SCOTT, 1919) makes it quite probable that one of these Cordaitean stems (*Mesoxylon multirame*) bore the seeds previously described by Mrs. ARBER (1910) as *Mitrospermum compressum*, but there is no indication that these seeds make a closer approach, either in their structure or in their mode of attachment, to those of the Pteridosperms, than do those of the other Cordaiteæ.

SUMMARY.

A description is given of the anatomy of *Acmopyle Pancheri*, Pilger, based chiefly on material from New Caledonia, the native habitat of the plant. Reasons are given for the view that the genus also occurs in the Fiji Islands.

So far as characters of generic rank are concerned, *Acmopyle* does not differ from *Podocarpus* in any of the following features: vegetative anatomy of root, stem and leaf; drupaceous character of the seed, megaspore membrane, female prothallus and young embryo, structure of male cone, microsporophyll, pollen-grain and probably male gametophyte. As is generally the case in *Podocarpus*, the seed is borne on a thick fleshy receptacle composed of the swollen axis and bracts.

The surface of the receptacle is tuberculate, and is raised into more or less prominent humps in the axils of the sterile bracts. The vascular supply to these axillary humps is in the form of paired strands inversely orientated with respect to the subtending bract-traces; the humps are therefore concluded to be vestigial axillary shoots, the entire receptacle being regarded as a reduced branch system. The vascular supply to the ovuliferous organ in the axil of the fertile bract does not differ, except in its much greater degree of development, from that to the humps in the axils of the sterile bracts; the ovuliferous organ is hence regarded as a brachyblast, and homologised with the ovuliferous scale of other Conifers.

Acmopyle differs from *Podocarpus*:

(1) In the nearly erect posture of the adult seed;

(2) In the complete fusion of the epimatium to the integument, even in the region of the micropyle, in the formation of which the epimatium takes part;

(3) In the much greater development of the vascular system of the seed, which forms a nearly continuous cup-like tracheal investment covering the basal two-thirds of the stone.

Taking into consideration the entire structure of *Acmopyle*, the genus is concluded to be the most highly specialised of the Podocarpineæ; there appears to be no support for the view that the family is a reduction series with *Podocarpus* as one of its most primitive genera.

The theoretical part of the paper also deals with other controversial questions, namely, the systematic position of the Taxineæ, the morphology of the ovuliferous

scale of Conifers, and the origin of the Conifers. With regard to the first of these questions, the conclusion arrived at is that the structure of the genera *Taxus*, *Torreya*, and *Cephalotaxus* is sufficiently distinct to justify their being placed in a separate phylum, for which the name Taxales is proposed, and which would be equivalent in rank and related to the Ginkgoales and Coniferales. The relations of the Taxales are, it is believed, closer with *Ginkgo* and the Cordaitales than with the Conifers, as here defined.

Concerning the ovuliferous scale of Conifers, the conclusion is in favour of the brachyblast theory.

No definite conclusion is expressed on the question as to whether the Conifers ultimately arose from microphyllous or megaphyllous (fern-like) ancestors, for, although the author confidently believes that they arose from Cordaitalean ancestors, the origin of the Cordaitales themselves from a megaphyllous (fern-like) stock is still considered to be "not proven" on the existing evidence.

Emphasis has been laid upon certain facts of a negative character, which are—pending further evidence—regarded as obstacles in the way of an unqualified acceptance of the megaphyllous theory. Of these facts, special importance is attached to a sharp differentiation that exists between those Gymnosperms (Cycadales, Pteridosperms) in which the seed is clearly borne upon a leaf, and those (Cordaitales, Ginkgoales, Taxales, Coniferales) in which it is either clearly borne directly upon an axis (Cordaitales, *Taxus*), or upon an organ which is probably some modification of an axis (Conifers, *Ginkgo*, etc.). For these two divisions of Gymnosperms, the author proposes the names Phyllosperms (with leaf-borne seeds) and Stachyosperms (with stem-borne seeds) respectively. It is argued that the Cordaitales, being Stachyosperms, cannot have arisen from Phyllosperm ancestors, unless there existed forms which bridge up this and other apparently wide gaps between the two divisions; and of the existence of these intermediate forms there is at present no evidence.

At the same time, it is fully recognised that the meagre evidence of a positive nature that is available at present, is distinctly in favour of a common megaphyllous origin for the Cordaitales and Pteridosperms.

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EXPLANATION OF PLATES.

PLATE 9. *Acmopyle Pancheri.*

Fig. 1.—Photograph showing habit. About $\frac{1}{6}$ nat. size.

Fig. 2.—Longitudinal section of young root-tubercle growing out into normal rootlet. Shaded cells in cortex forming an ill-defined infected zone. About × 17.

Fig. 3.—Transverse section of young diarch root. Hoop-like thickenings on inner cortical cells, in section appearing like beads. About × 45.

Fig. 4.—Transverse section of leaf. Compare text-fig. 2, A. *x.*, xylem; *p.*, phloem; *c.*, mucilage canal. About × 28.

Fig. 5.—Section of leaf cut parallel to dorsal surface, showing mosaic of large thin-walled mucilage cells and small crowded palisade cells. About × 100.

Fig. 6.—Section of leaf cut along plane of expansion, showing accessory transfusion tissue. The vascular bundle of the leaf lies not far from the lower edge of the photograph. The upper edge of the photograph shows the leaf margin. About × 25.

Fig. 7.—Camera-lucida drawings of stomata. About × 375.

a. Section of leaf cut very nearly parallel to stomatal surface. The stoma towards the lower end of the series was cut very near the cuticle, and shows the deep-staining dorsal lignified lamellæ, and the thickenings of the septa between the guard-cells. The stoma at the upper end was sectioned along a plane midway between the dorsal and ventral lamellæ. The sinuous lines over the septal thickenings are cellulose layers. The walls of the epidermal cells show moniliform thickenings; the cell-contents have been omitted. There is usually one polar subsidiary cell common to two consecutive stomata, and 2–4 lateral subsidiary cells to

each stoma. The lateral subsidiary cells of each stoma touch those of the stomata in front and behind.

b. Transverse section through middle of stoma, showing dorsal and ventral lignified lamellæ.

c. As we proceed towards the end of a stoma (fig. 7, *c*), the ventral lamellæ gradually vanish, their place being taken by the median septal thickenings; the dorsal lamellæ here stand almost perpendicularly instead of almost horizontally as in the middle region of the stoma.

d. Nearly median longitudinal section of stoma. Of the lignified portions only the septal thickenings are seen, besides the lining of the stomatal pore.

Fig. 8.—Median longitudinal section of microstrobilus. ×, organic apex of axis; ✱, peltate terminal stamen, the sterile "shield" forming a (?) protective cap. About × 17.

Fig. 9.—Transverse section of male cone axis, with well-defined epidermal layer of "resin"-cells, and ring of mucilage canals round a circle of strands. The light patches in the canals are coagulated masses of mucilage, one of which contains a deep-stained tabular hexagonal crystal. About × 40.

Plate 10. *A. Pancheri.*

Fig. 10.—Transverse section of stamen. ××, points of dehiscence; the figure 10 points to the mucilage canal of the sporophyll strand; the strand itself is not preserved in this section. On the abaxial (lower) side there is a ⅄-shaped air space between the sporangia. About × 32.

Fig. 11.—The same, further enlarged to show reticulate markings on the wings of the pollen-grains, and the thin septum between the sporangia. About × 175.

Fig. 12.—Male inflorescences. Natural size.

Fig. 13.—Transverse section of outer cortex of a receptacle, showing the thick cuticle covering the rounded tubercles; the large clear mucilage cells, and smaller "resin"-cells. About × 35.

Fig. 14.—Part of transverse section of a receptacle, showing the trace of a sterile bract (to the right of the black cross), and two smaller inverted strands (left of the cross) destined to supply an axillary hump. About × 35.

Fig. 15.—Ventral view of stone. The large white spot near the lower end is the eccentric chalaza. The ventral loop of the "escarpment" is not far from the chalaza. About × 3.

Fig. 16.—Dorsal view of stone. The chalaza is invisible from this side, and the dorsal loop of the "escarpment" is nearer the micropyle than it is in fig. 15. About × 3.

Fig. 17.—Basal view of stone, showing the eccentric chalaza and the radiating marks corresponding to the strands in the flesh. About × 3.

Fig. 18.—View of stone from the left side. One arrow points towards the chalaza, the other away from the micropyle. A curved faintly prominent ridge connects the two points. Note the relative positions of the two loops of the "escarpment" with respect to the chalaza. About × 3.

Fig. 19.—Apical view of stone. The micropyle is just visible as a minute black spot in the centre. About × 3.

PLATE 11. *A. Pancheri.*

Fig. 20.—Part of transverse section of seed, at about the level of text-fig. 32 (compare index-figure on p. 275). *o.o.f.*, outer zone of outer flesh; *i.o.f.*, inner zone of outer flesh; *scl.*, sclerotesta; *cup.*, vascular cupule; just inside this point a strand is seen coming off from the inner face of the cupule.

The horizontal line indicates the plane of dehiscence of the stone. Note that the ventral (upper) valve is considerably larger than the dorsal. The arrow points to one of the carinæ of the sclerotesta. About × 5½.

Fig. 21.—Transverse section of seed, cut at about the level of text-fig. 32 (see also text-fig. 35). The vascular cupule is seen in section as a faint white line with moniliform thickenings. Slightly larger than natural size.

Fig. 22.—Megastrobilus. *a.* Oblique dorsal view of two strobili on a forked, strongly curved peduncle. The curved ridge on the back of the seed, the tuberculate surface of the receptacle with the sterile bracts, and the broad fertile bract wedged in between the receptacle and seed, are clearly seen. Nat. size.

b. Ventral view of a strobilus attached to a pectinate shoot. In the natural position of the organs, if the shoot is seen from the upper side (as in our photograph) the fruit would be concealed beneath it. In order to expose it to the view, the fruit has been moved out of its natural position. The sub-apical micropyle is well seen, but the curved ridge is invisible from this side. Nat. size.

c. Median longitudinal section of fruit. Nat. size.

Fig. 23.—Ventral view of a megastrobilus, showing (1) the large round hump on the receptacle, with the most distal sterile bract projecting from its centre (just below the seed); and (2) three other sterile bracts (two right and left and one in the middle below), each with an axillary hump. Nat. size.

Fig. 24.—View from left side of a seed from which the outer zone of the outer flesh was shaved off so as to expose the vascular cupule, stiffened by a network of strands. The two basal strands are also exposed. Comparison with Plate 2, fig. 18, shows that the cupule does not extend beyond the "escarpment."

The seed was photographed while immersed in alcohol, for then the xylem appeared white and showed up well against the "resinous" inner zone of the flesh. Slightly larger than natural size.

Fig. 25.—The dome-shaped free portion of a nucellus, flattened out under a coverslip after removal of the nucellar cone, of which the scar is indicated by an arrow. Round this scar is a broad areole of light colour, which contrasts well with the dark peripheral part rich in "resin"-cells. About × 20.

Fig. 26.—Apical part of megaspore, with a pro-embryo to one side of the axis. The coiled suspensor tubes are seen cut in different planes. The apex of the megaspore is produced into a blunt process which may (?) be likened to the "tent-pole" of *Ginkgo*. About × 11.

Fig. 27.—Drawings to show the different types of tracheides in the vascular cupule of the seed.

Fig. 28.—Superficial cells of megaspore, with poorly developed megaspore membrane, and anticlinal walls specially thickened next to the membrane.

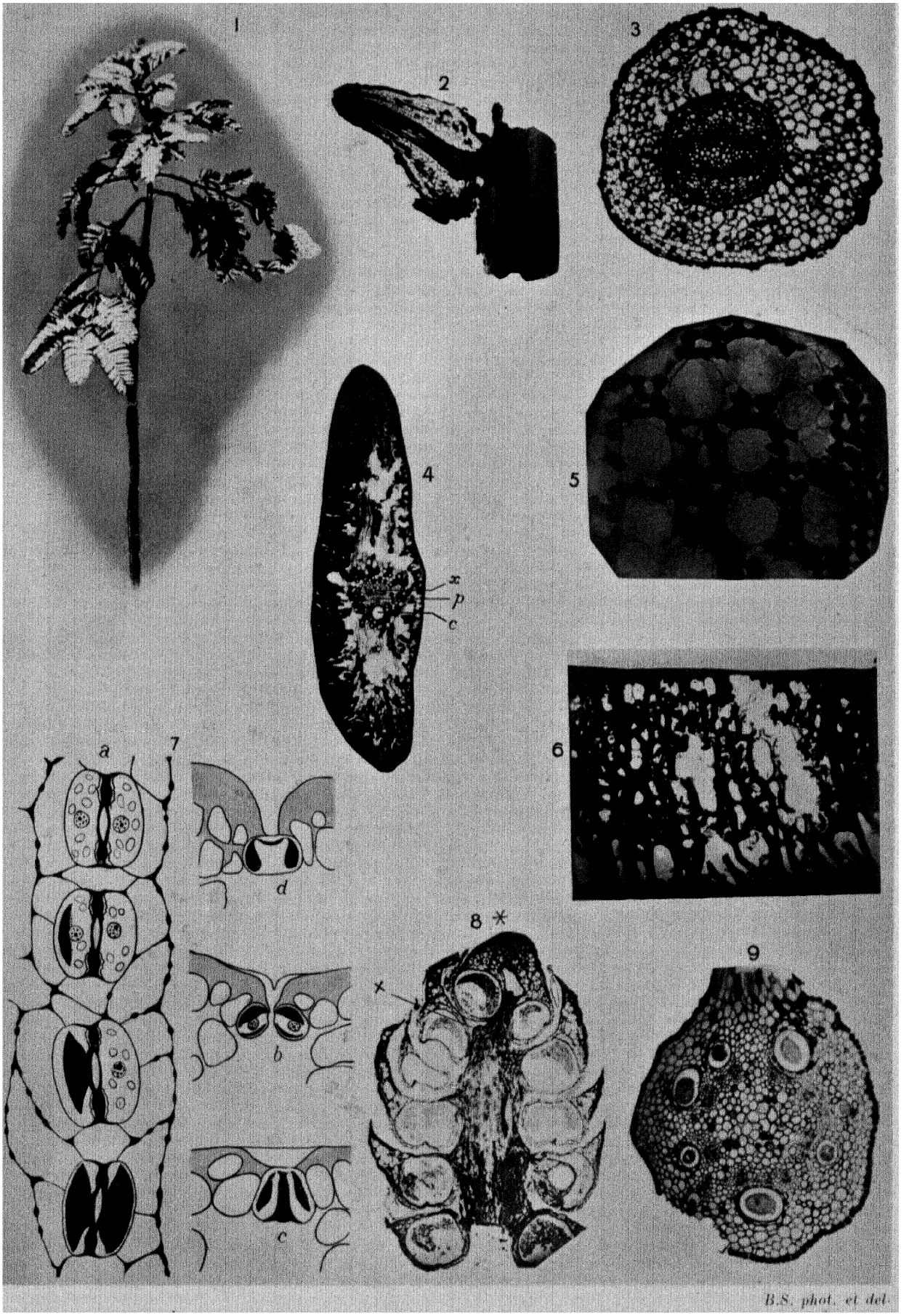
Fig. 29.—Surface view of superficial layer of megaspore. The multinucleate cells have their thickened anticlinal walls perforated by straight pits which impart a prismatic appearance to the walls.

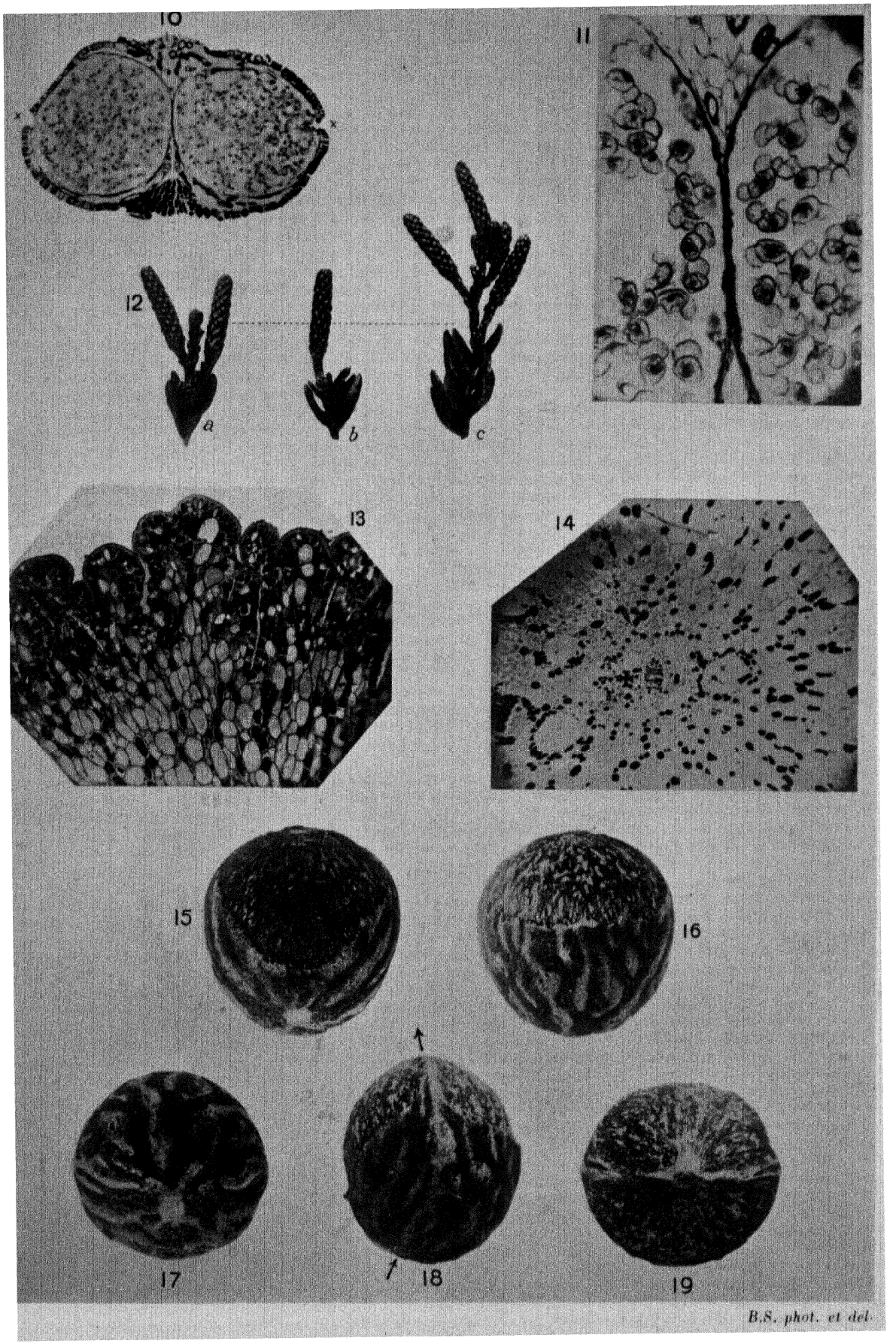
Podocarpus imbricatus.

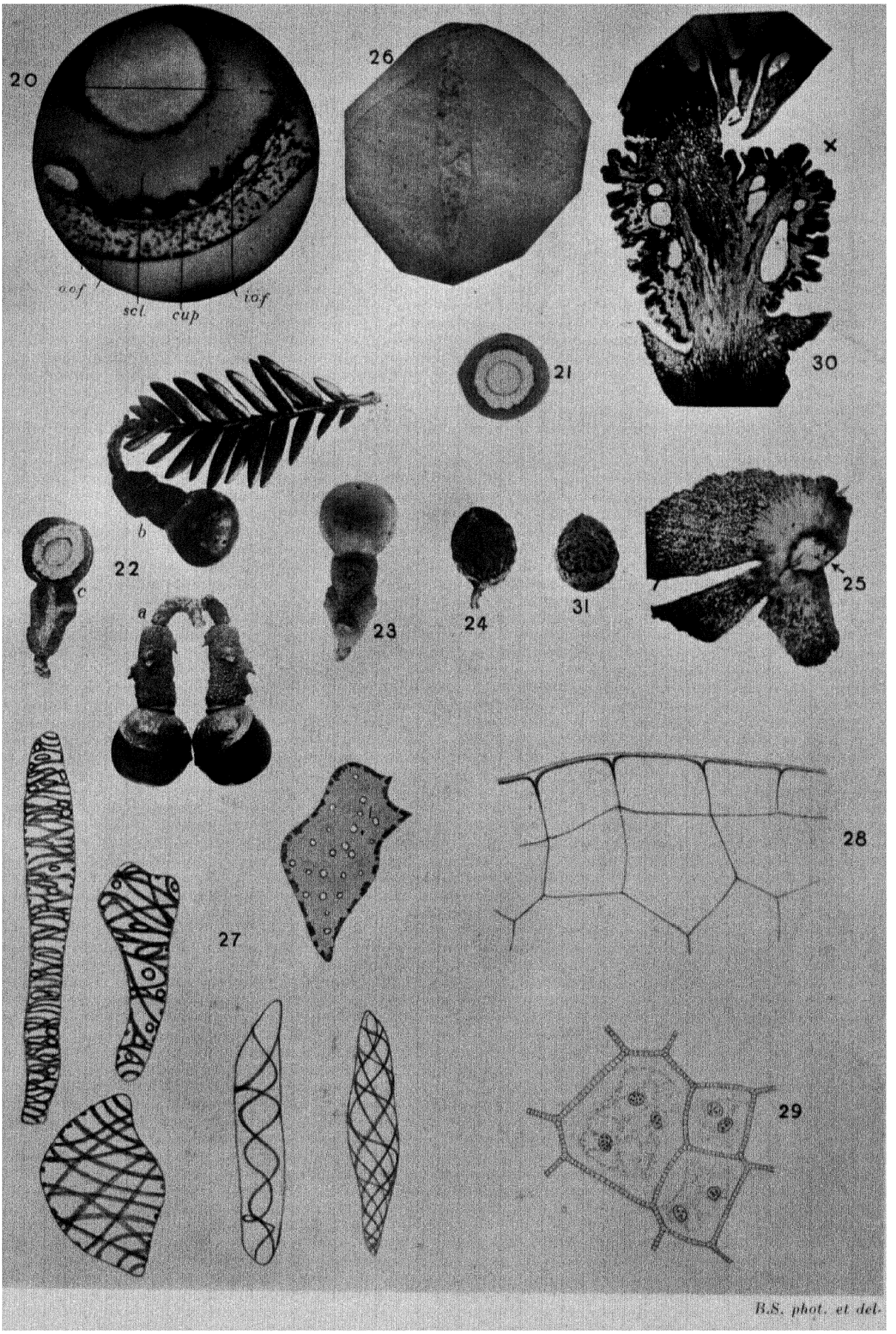
Fig. 30.—Longitudinal section of receptacle and micropylar region of seed. As in *Acmopyle* the receptacle has a tuberculate surface (compare Plate 11, figs. 22*a*, 23). At × there was a sterile bract (in a position corresponding to that of the last sterile bract of *Acmopyle*, see Plate 11, fig. 23), but this was removed before the section was prepared. About × 17.

Podocarpus sp.

Fig. 31.—Stone of a new species discovered by COMPTON in New Caledonia. The sculpturing shows a general similarity to that in *Acmopyle* (see Plate 10, fig. 15). Slightly enlarged.


1
2
3
4
x
p
c
5
6
7
a
d
b
c
8 *
x
9
B.S. phot. et del.


10
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19
B.S. phot. et del.


20
a.o.f.
scl.
cup
i.o.f.
26
30
x
21
22
b
c
a
23
24
31
25
28
27
29
B.S. phot. et del.

VIII.—*On the Calcification of the Vertebral Centra in Sharks and Rays.*

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Communicated by Prof. E. W. MacBride, *F.R.S.*

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Introduction.

In this paper are recorded the results of an investigation undertaken at the instance of Dr. A. Smith Woodward for the purpose of ascertaining to what extent the pattern presented by the calcified laminæ of the centrum is of value as an aid to the classification of Elasmobranch fishes, and to the identification of vertebræ found in the fossil state. The subject was dealt with exhaustively in 1879–1885 by Hasse, who, in his monograph 'Das natürliche System der Elasmobranchier,' claimed that the differences in the disposition of the calcified laminæ in the various genera and families of Elasmobranchs occur with such constancy and regularity that they may be accepted with confidence as an important factor in taxonomy. During the years, however, that have passed since the publication of this monograph the thesis has come to be looked upon with suspicion, and vertebrate morphologists at the present time do not, as a whole, regard Hasse's definitions of the Cyclospondyli, Tectospondyli, and Asterospondyli as consistently applicable to the genera and species included by him within those groups.

The material studied in the course of the investigation was to a large extent accumulated several years ago (see p. 313), and it was only the superior attraction of *Cephalodiscus* as a subject of research that prevented the work from being brought to an earlier conclusion. The examination of this accumulated material, and of that more recently acquired, was carried on in the Huxley Research Laboratory of the Imperial College of Science during the winter of 1917 and from May, 1919, to May, 1920, and I hereby acknowledge my great indebtedness to Prof. E. W. MacBride and the administrative officers of the College for the facilities offered there for the prosecution of the work. I have further to thank Prof. MacBride for frequent advice and for valuable suggestions made during the progress of the research. My thanks are also due, and are hereby tendered, to Dr. A. Smith Woodward and Mr. C. Tate Regan, of the British Museum (Natural History), for many helpful hints and suggestions. Acknowledgments and thanks for material kindly furnished by various donors are recorded on p. 313.

Material Studied.

Owing to an indefiniteness that prevails in the scope of the term Elasmobranchii, it may here be explained that the fishes studied in the course of the present investigation comprise the ordinary sharks, dog-fishes, monk-fish, skates, and rays, but do not include the Holocephali, nor the Cladodont, Acanthodian, and Pleuracanth fishes.

The fishes are those embraced within Regan's Order 4, Euselachii (**29**, p. 722), and the classification of the fishes here adopted is the one propounded by him in the paper quoted. The convenient expression "Batoid fishes" (=Batoidei of Günther), as used in the following pages, is equivalent to the Hypotremata of Regan; it includes the Torpedinidæ, Rhinobatidæ, Raidiiæ, and Dasybatidæ, and excludes *Squatina* and

Pristiophorus, which two genera were associated by HASSE with the ray-like fishes. This is the sense in which REGAN uses the term Batoidei on p. 737, although in his classificatory table (p. 724) the Division Batoidei excludes the electric rays.

In the course of the investigations 151 fishes were examined, belonging to 44 genera and 68 species. Accepting REGAN's classification of 1906 (**29**), they may be tabulated as follows :—

Division.	Family.	Specimens examined.	Genera.	Species.
Notidanoidei	Chlamydoselachidæ	0	0	0
	Hexanchidæ	2	1	1
Galeoidei	Odontaspididæ	3	2	3
	Lamnidæ	7	4	4
	Orectolobidæ	9	4	4
	Scyliorhinidæ	19	2	5
	Carchariidæ	21	6	10
Squaloidei	Cochliodontidæ	0	0	0
	Hybodontidæ	0	0	0
	Cestraciontidæ	8	1	1
	Squalidæ—			
	Squalinæ	22	7	9
	Pristiophorinæ	4	2	4
	Squatinidæ	4	1	1
Narcobatoidei	Torpedinidæ	12	3	4
Batoidei	Rhinobatidæ—			
	Pristinæ	5	1	3
	Rhinobatinæ	7	3	5
	Raiidæ	17	1	5
	Dasybatidæ	11	6	9
Total		151	44	68

The material used in the present investigation was brought together in a variety of ways. Much of it was accumulated during the years 1903–1907, when I was interested in the study of fishes owing to the fact that I was then assisting Sir E. RAY LANKESTER in rearranging the exhibited series of fishes in the British Museum (Natural History). Most of the sketches and notes made during those years were based upon specimens contained in the Museum collection. Further material was subsequently obtained by purchase in the markets of Grimsby, Folkestone, and other coast towns, and in Billingsgate Fish Market, London, and some was purchased from the Marine Biological Laboratory, at Plymouth. My acknowledgments are due to Mr. J. HORNELL for material from India during 1907–1910, to Prof. W. N. F. WOODLAND for specimens from Naples, and to Prof. W. A. HASWELL for some from Sydney.

More recently I have had the advantage of examining selected pieces of vertebral column excised from young and embryo fishes, owing to the kindness of Mr. C. TATE REGAN, Assistant Keeper in the Department of Zoology at the British Museum (Natural History). To him I offer my cordial thanks. For the use of other material

in recent months I have to thank Prof. E. W. MacBride, of the Imperial College of Science, Prof. J. P. Hill, of University College, London, Prof. E. S. Goodrich, of Oxford, and Mr. R. H. Burne, of the Museum of the Royal College of Surgeons.

None of the material examined was specially fixed with a view to studying the histological detail. Pieces of the vertebral column of fresh specimens of some of the adult fishes might have been so fixed, but it was not considered necessary. Where there really was need for a study of fine histological structure, in the late embryos and young fish, special measures of fixation and preservation were not possible; these specimens proved to be so difficult to obtain that one was grateful to secure and examine whatever was available, regardless of its histological condition. The only exceptions were *Scyliorhinus canicula* and *Squalus acanthias*, which happen to be among the least interesting of the forms under consideration.

For checking the observations on the incipient activity of the perichondrium, referred to on p. 330, it is eminently desirable that specially prepared material should be examined when the opportunity offers. *Galeus canis*, of a length of 230 mm., would be suitable; also *Stegostoma tigrinum*, 210 mm. long, *Chiloscyllium plagiosum*, 120 mm. long, *Rhinobatus granulatus*, 233 mm. long and 82 mm. broad, *Trygonorhina fasciata*, 265 mm. long and 108 mm. broad, and post-cloacal vertebræ of a *Myliobatis*, measuring about 90 mm. in breadth.

The use of the generic and specific names as adopted by Regan (**29**) in conformity with accepted rules of priority in nomenclature necessitates the discarding of many names that have long been familiar to anatomists and embryologists. Lest such workers may not, in the alphabetical list of the genera and species of the fishes studied, recognise at once the fishes under the names that are in use at the British Museum (Natural History), I have given in brackets the more familiar names, or at all events the names which to me are more familiar, thus: *Scyliorhinus stellaris* [= *Scyllium catulus*]; *Somniosus microcephalus* [= *Læmargus borealis*].

Alphabetical List of Genera and Species of Fishes Examined.

Alopias vulpes.

Carcharias laticauda.
„ sp.
„ *walbeehmi.*
Carcharodon rondeletii.
Centrophorus calceus.
„ *squamosus.*
Cestracion philippi.
Cetorhinus maximus [= *Selache maxima*].
Chiloscyllium plagiosum.

Dasybatis [= *Trygon*] sp.
„ *thalassia.*
Echinorhinus spinosus.

Galeus canis.
Ginglymostoma cirratum.

Hemigaleus balfouri.
Heptanchus cinereus.
Hypnos subnigrum.

Lamna cornubica.

Mustelus vulgaris.
Myliobatis aquila.
„ *nieuhofi.*
„ sp.

Narcine brasiliensis.

Odontaspis americanus.
Orectolobus [= *Crossorhinus*] *barbatus.*
Oxynotus centrina [= *Centrina salviani*].

Pliotrema warreni.
Pristiophorus cirratus.
„ *japonicus.*
„ *nudipinnis.*
Pristis cuspidatus.
„ *pectinatus.*
„ *perrotteti.*
Pristiurus melanostomus.
Pteroplatea micrura.

Raia batis.
„ *clavata.*
„ *maculata.*
„ *marginata.*
„ *punctata.*
Rhinobatus granulatus.
„ *halavi.*
„ *undulatus.*
Rhinoptera javanica.
Rhynchobatus djeddensis.

Scapanorhynchus lewisi.
„ (*Mitsukurina*) *owstoni.*
Scyliorhinus analis [= *Scyllium anale*].
„ *canicula* [= *Scyllium canicula*].
„ *marmoratus* [= *Scyllium marmoratum*].
„ *stellaris* [= *Scyllium catulus*].
Scymnorhinus [= *Scymnus*] *lichia.*
Somniosus microcephalus [= *Læmargus borealis*].
„ *rostratus* [= *Læmargus rostratus*].
Sphyrna [= *Zygæna*] *blochii.*
„ „ sp.
„ „ *tudes.*
Spinax niger.
Squalus acanthias [= *Acanthias vulgaris*].
Squatina squatina [= *Rhina squatina*].
Stegostoma tigrinum.

Torpedo hebetans.
„ *narce* [= *ocellata*].
Triacis semifasciatus.
Trygonorhina fasciata.

Urogymnus asperrimus.
Urolophus (*Trygonoptera*) *testaceu.*

Further particulars respecting the material studied are given at the beginning of each of the various sections of the chapter headed "Detailed Observations" (pp. 347–394).

Methods of Investigation.

The subject lends itself to investigation by four different methods :—a comparison of the structural features of the vertebræ in the adults of a large range of closely allied genera and a contrast between the vertebræ of genera that are more remotely related, a comparison of the vertebræ of the caudal, cloacal and trunk regions of the same individual fishes, a comparative study of the vertebral characters in embryos, young, and adults of certain selected species, and a tracing of the lines of evolution by a study of the vertebræ of extinct forms in conjunction with those of their nearest modern allies. The methods might be termed respectively the morphological, individual, developmental and palæontological methods. Hasse adopted mainly the first and last of the four, without, however, entirely neglecting the developmental method.

The last, or palæontological, has not been followed in the present inquiry, although the method is not without its value. It is of considerable interest, for instance, to note that in Jurassic forms of *Squatina* there are fewer calcified layers than in the existing species, and that in fossil Notidanids the centra are calcified to a greater

extent than are those of *Heptanchus cinereus*. But the method exposes one to the risk of the fallacious process of arguing in a circle, for the taxonomic position of an extinct shark is to some extent decided by the features of the calcified parts of its vertebræ. *Palæospinax*, for instance, now regarded as allied to the Cestracionts, was when first discovered regarded as a Squalid because of the simplicity of its vertebræ. The identification of the fossil vertebræ, again, may possibly be at fault. It is extremely probable that the vertebra figured by HASSE as that of a *Crossorhinus* from the Gault of Cambridge (**15**, D, Plate 25, fig. 10) is not an Orectolobid at all; it is much more likely to be a Lamnid (*cf.* Plate 27, figs. 26 and 37).

The first method of inquiry mentioned above, that of the comparative study of the vertebræ of adult fishes, was largely followed by HASSE, and the bulkiness of his monograph is rather to be explained by this fact. In justice to him it may be pointed out that until the vertebræ of any particular fish are examined one cannot decide whether they are of interest or not; when the examination has been made, the author is performing a service to science by placing his observations on record, for even if the structures prove to have no very direct bearing on the elucidation of the problem, he spares his successors the labour of an independent examination of the vertebræ. Negative results, though disappointing from the author's point of view, are not without their value as an addition to knowledge.

The morphological method of inquiry, however, as it was pursued by HASSE, fails to carry conviction owing to his neglect of the second or individual method; he contrasted the trunk vertebræ, for instance, of one species with the caudal vertebræ of another without, in some cases, at all events, first ascertaining the extent of the differences that exist between the vertebræ of the trunk and caudal regions in these individual fishes. The method, also, is not of great service if adopted independently of the developmental method, and it is only in the Batoid fishes, where the membrana elastica externa disappears early, and the developmental method largely fails in consequence, that one is glad to fall back upon it as the main line of research.

Concerning the developmental method, but little need be said here, in view of the considerable detail in which the subject is treated in the chapter headed "Development of the Centra" (pp. 323–334). Suffice it to say that the study resolves itself largely into a delimiting of the sheath-cartilage by a diligent search for the remnants of the membrana elastica externa at as late a stage of development as possible. In some cases these remnants persist in the vertebræ of adult fishes (*Cestracion*, *Mustelus*), in others they disappear very early (*Torpedo*). The study of the mode of origin of certain constituent parts of the centrum by the generative activity of tracts of perichondrial connective tissue also comes within the scope of this part of the inquiry.

In some cases, it is to be noted, the results obtained by this method are rather at variance with those obtained by the "individual" method of research. In *Cetorhinus*, for instance, the hinder caudal vertebræ of the adult fish give a better indication of

Lamnid structure than do the trunk and anterior caudal vertebræ of the young fish (fig. 14, C, p. 361 ; fig. 13, A, p. 360 ; and 15, D, Plate 32, fig. 3). Similarly, the hindermost caudal vertebræ of *Pristis* and *Rhynchobatus* exhibit a calcified four-rayed star, which is more Batoid in character than the gradually thickening "investing layer" around the double cone which is seen in trunk and anterior caudal vertebræ of late embryos (fig. 31, E, p. 385 ; fig. 28, A, p. 382).

The "individual" method has much to recommend it, and it is quite possible to conceive that the study of the whole vertebral column in a few appropriately chosen fishes may be more productive of results of morphological value than the study of isolated vertebræ taken one from each of a much greater number of species, particularly if these single vertebræ are not taken from corresponding regions of the body, so as to render the comparison just. One of the results of the present inquiry has been to show that in a range of closely allied forms the vertebræ of the caudal region differ less than do those of the trunk, and they are more elementary in structure ; witness, for instance, the series of *Raia*, shown in fig. 33, p. 388. A series of *Torpedo*, *Rhinobatus*, or *Cestracion* would illustrate the same fact.

It is not a new thesis that the structure of the caudal vertebræ of sharks and rays is simpler than that of the vertebræ of the trunk region. KÖLLIKER, in 1864, wrote : "Die vorderen Wirbel von *Ginglymostoma* sind verwickelter gebaut als die des Schwanzes" (20, p. 53). And GADOW and ABBOTT write : "the tail of embryonic Elasmobranchs generally shows more primitive conditions or stages than the trunk," and "as the tail generally shows earlier stages than the trunk, a forward gradation can be made out in each embryo in passing from the tail towards the head" (6, pp. 181 and 178).

The general proposition, however, that the caudal vertebræ are in a less advanced stage of evolution than the trunk vertebræ is not universally accepted, for GRAHAM KERR, in his text-book of Embryology (London, 1919, p. 294) writes, that in sketching out the development of the vertebral column : "The assumption will be again made use of, as it was in dealing with the mesoderm segments, that the trunk region has in all probability departed least from the primitive conditions . . . Some writers will be found to assume that the caudal region is more nearly primitive." KERR'S conclusion is based upon the fact that the Vertebrate is essentially a cœlomate animal, and that the existence of a tail-region devoid of splanchnocœl is secondary. But while, as regards the general evolution of the vertebrate animal, few will deny that the anus was primarily posterior, and that the caudal appendage appeared as a later growth, the axial skeleton of the primitive vertebrate—as Prof. MACBRIDE has been good enough to point out—probably remained notochordal for a considerable time after the evolution of the tail, and any differences in the structure of the vertebræ of the caudal and trunk regions arose comparatively recently in a vertebral column that was, despite this late origin of the tail in comparison with the trunk, for a long time more or less uniform throughout.

At one period of the inquiry it seemed as though the hindermost caudal vertebræ, owing to their simplicity, might afford a clue to the significance of any substantial differences that might be observable in the trunk vertebræ of two species of fish. The hind caudal vertebræ of such two fishes being the same, one would only have to study the transition forwards in the two cases to arrive at the complete explanation of the differences. But, while some interesting results have been arrived at, the complete solution of the problem by the employment of this method fails, in consequence of the pattern in the hinder caudal vertebra becoming, in many cases, not simpler, but irregular and unreliable.

Among the few cases in which the irregularity is but little marked, one might mention *Raia*. As one passes backward in the caudal region, the longitudinal lamellæ, horizontal and vertical, become smaller and smaller and finally disappear, leaving only a double cone, long in proportion to its width, but not otherwise remarkable. Still further back, the double cone becomes a granular tube with its anterior and posterior ends enlarged.

Another interesting case is that of *Pristis*, previously alluded to. Almost all the vertebræ have a bulky, compact secondary calcification, of circular outline, but in the hindermost caudal vertebræ (fig. 31, E, p. 385) the circle gives place to horizontal and vertical rays, similar to those in less aberrant Batoids (*Raia*, fig. 32, E, F, p. 387; *Rhinobatus*, fig. 30, D, p. 383).

Yet another case worthy of mention is that of *Somniosus microcephalus* [= *Læmargus borealis*]. This shark is generally regarded as having no calcification in its vertebral column. But in the terminal vertebræ of the tail there are some five or six calcified, granular tubes with dilated ends, closely resembling the elongated double cones of *Raia* (*supra*), except in being larger. Applying the argument set out above, one concludes that the majority of the vertebræ of this shark, in having no calcification, are *more degenerate* than these few caudal vertebræ; and the conclusion is strengthened by the fact that in another species of the genus, *Somniosus rostratus*, the vertebræ are as typically Squaline as those of *Scymnorhinus*, there being well calcified double cones throughout the vertebral column.

This case naturally leads on to that of *Heptanchus*, in which the vertebræ of the middle part of the caudal fin have double cones and radiating secondary lamellæ, but as one passes forwards these gradually dwindle. The fact that the structure of the caudal vertebræ is more complex and more perfect than that of the trunk vertebræ does not tell against the argument; the conclusion to be drawn is that the trunk vertebræ are more advanced, along a line of degeneration, than the caudal (see p. 349). Pursuing the argument further, one would say that the vertebral column of *Hexanchus* is still more degenerate, for not only the trunk vertebræ, but the caudal vertebræ as well have no calcified structures.

It is argued elsewhere (pp. 328 and 362) that the comparative simplicity of the centra of *Scyliorhinus canicula* and *Scyliorhinus stellaris* is due to a reduction of

intermedialia that are so well marked in *Scyliorhinus marmoratus*, and it is not beyond the bounds of possibility that the vertebral structure of the Squalinæ is of the same origin. The hindermost caudal vertebræ of *Scyliorhinus canicula*, *Scyliorhinus stellaris*, *Squalus acanthias*, and *Scymnorhinus lichia* were therefore carefully studied, in order to ascertain if in them the intermedialia are better developed than in the more anterior parts of the vertebral column; but the search proved abortive. Similarly, the hindermost vertebræ of *Squatina* show no departure from the type of structure characteristic of the genus; the rings diminish in number as one passes backward, but the pattern in the hindermost centra is still that of concentric rings.

Technique.

Small vertebræ, those of embryos, young fish and small adults, were decalcified and cut into serial sections by the ordinary paraffin-embedding method. The best results were obtained by decalcifying with a 3 per cent. solution of nitric acid in 90 per cent. alcohol, used for a period of about three weeks, more or less, according to the degree of calcification of the vertebræ.

The most suitable thickness for the sections is 0·008 mm. Since a piece of vertebral column consisting of three or four vertebræ yields a very long ribbon, those sections alone were mounted which pass through the middle of the length of the centrum; the ribbon was searched, with the aid of a lens, for those sections in which the ring of the double cone is of minimum size.

For distinguishing calcified (now decalcified) parts from those composed of uncalcified cartilage the stain found to give the best results was EHRLICH'S hæmatoxylin fluid, saturated with Orange G, and diluted with twice its bulk of distilled water. In slides left in this solution for half an hour the calcified parts are stained deeply and the cartilaginous parts feebly.

For distinguishing the remains of the membrana elastica externa, upon which so much depends in an inquiry of this kind, the best results were secured by staining the sections for 10 minutes in a 0·5 per cent. solution of Congo Red in 90 per cent. alcohol, a method which is more expeditious than the former, since it is not necessary to pass the slide through the graded alcohols down to water, and then back again after staining.

As a general routine practice both of these methods were employed, portions of the same ribbon being stained, some with the former and some with the latter fluid. Bismarck Brown, a stain recommended by HASSE, was found to be less satisfactory than Congo Red. Eosin, Saffranin, Magenta, and Carmine stains were also tried, but discarded as being less efficient than those mentioned above.

For detecting the remains of the membrana elastica externa in difficult cases the best results are obtained by using a fairly high-power objective (4 mm.), and nearly closing the substage diaphragm. It is not always easy to distinguish between a small

remnant of the membrana elastica externa and a flattened cartilage-cell, but as a rule the latter differs from the former in being surrounded by a clear space, owing to the shrinkage of the cell within the lacunar space in the matrix which it previously filled. There is no occasion to be discouraged by the fact that in some particular section the remnants of the membrane cannot be recognised; it not uncommonly happens, in *Scyliorhinus canicula*, for instance, that such remains are to be seen in one vertebra and not in that which immediately follows.

Large vertebræ, those more than 10 mm. in diameter, were cut with a very fine saw (dentist's ribbon-saw, with 25 teeth to the centimetre), and the thin slice was then ground under water upon a carborundum stone, and finished upon a razor-hone or water-of-Ayr stone. When thin enough to be transparent, the section was stained and mounted exactly like a section cut by the paraffin method. Sections of large dried vertebræ can be ground on glass-paper.

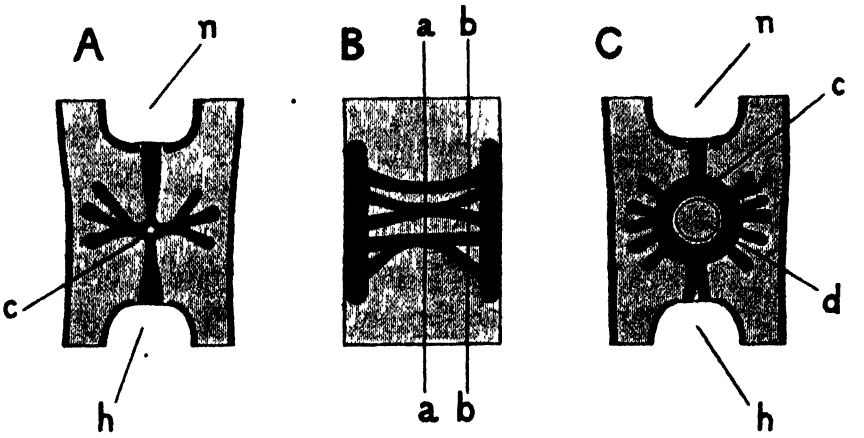


FIG. 1.—*Raia batis*, post-cloacal vertebra, × 2. Figures illustrating how the pattern of a transverse section taken near the end of a centrum differs from one taken through the middle of its length. The upper part of the neural arch and the lower part of the hæmal arch are not shown. A, transverse section of the vertebra cut midway between the anterior and posterior ends of the centrum, in the direction of the line *aa*. B, side view of the vertebra, after removal of the superficial calcification. The details of the arch-cartilages are not shown. C, transverse section of the vertebra taken near the end of the centrum, in the direction of the line *bb*; *c*, primary double-cone calcification; *d*, remains of the notochord; *h*, hæmal canal; *n*, neural canal.

In the case of large vertebræ a great deal can be learnt as to the disposition and relations of the various calcified parts by removing the uncalcified cartilage by means of a 5 per cent. solution of hot caustic soda. It is advisable not to carry the treatment too far, or the resulting structure will crumble; the aim is, not to dissolve the cartilage, but to soften it sufficiently to allow of its easy removal by steel instruments or a stiff brush.

For adequate comparison of one vertebra with another the most suitable sections are, as already pointed out, those cut through the part where the double cone is smallest; sections cut otherwise than through the middle of the length of the centrum are difficult to interpret, and except for the determining of special points of structure,

are rather to be avoided. KÖLLIKER gives a figure of a *Galeus* vertebra cut towards the end of the centum (**19**, Plate 2, fig. 12, 3), and DANIEL gives two of *Cestracion* ('Journ. Morph.,' 26, 1915, Plate 6, figs. 19 and 22).

Transverse sections through the same vertebra present different patterns according to their relative distances from the middle, and in the case of vertebræ with radiating lamellæ the rays are usually found to be more numerous in the sections taken near the end of the centrum than in that through the middle of its length (*cf.* C and A in fig. 1, p. 320). This difference is due, as will be seen by reference to a side view of the centrum (fig. B; see also HASSE, **15**, D, Plate 30, fig. 29, *Carcharodon*), to a splitting of the lamellæ into two near the anterior and posterior ends. The increased size of the cone-section also makes a profound difference in the appearance.

TERMS EMPLOYED.

As a general rule in the following pages the vertebræ under consideration are described as selected from some particular region of the body, *e.g.*, branchial, mid-trunk, pre-cloacal, cloacal, post-cloacal, anterior caudal, mid-caudal, posterior caudal; but in many cases the region is indicated with more precision by reference to the fins, thus: "in front of the second dorsal fin," "above the anal fin," "halfway along the caudal fin."

The sheath-cartilage is that part of the definitive centrum which is developed from the chondrified sheath of the notochord (see p. 323). Its inner and outer limits respectively are marked by the membrana elastica interna and the membrana elastica externa. The sheath-cartilage differentiates early into three zones, the inner, middle and outer.

The term "double cone" has so long been in common use that its definition might be deemed scarcely necessary; but although in strict terminology the double cone is the calcified middle zone of the sheath-cartilage, the structure which on isolation from the vertebra is called the double cone possesses an "investing layer" (Belegschicht) formed from the calcification of the innermost part of the outer-zone cartilage. The dried double cones of *Scymnorhinus* and *Oxynotus*, as drawn in fig. 2, possess this

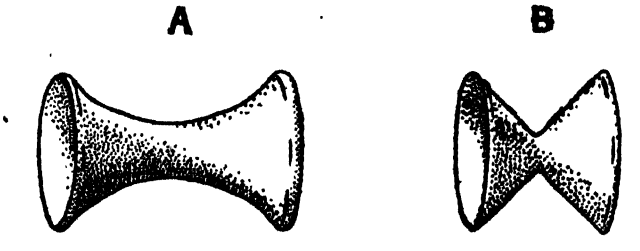


FIG. 2.—Double-cone calcification after removal of all cartilaginous parts. A, *Scymnorhinus lichia*, × 1·5. B, *Oxynotus centrina*, × 2.

investing layer; if the latter were scraped away, the double cone would be too fragile to handle. In descriptions of transverse sections, nevertheless, the term

double cone is used in the strict sense—the part marked *c* in the text-figures is the calcified middle-zone cartilage, and the investing layer is separately marked *i l*.

The term arcualia is adopted from the paper by GADOW and ABBOTT (**6**) as meaning the arch-cartilages, *i.e.*, the cartilages of the neural and hæmal arches. The term intermedialia is a new one and refers to components of the centrum that are situated between the arcualia, either between the two basidorsal cartilages, or between the two basiventrals, or between a basidorsal and a basiventral cartilage. The intermedialia, as explained on p. 328, are products of the activity of the superficial perichondrium, and they make their appearance long after the arcualia are definitive structures.

Radiating lamellæ are longitudinally disposed calcified plates that lie in radial planes between the double cone and the surface of the centrum; seeing that in the majority of instances these are observed in transverse sections of the vertebræ, in which they appear as the rays of a star, the term "ray" is frequently employed instead of radiating lamella. Similarly, in the case of *Squatina*, the calcified lamellæ are really in the form of coaxial tubes, but by a pardonable licence in expression these are frequently spoken of as concentric circles or rings, since that is the appearance that they present in transverse sections.

The word diagonal is used as the equivalent of the German "schräg" in referring to those lamellæ which occupy an oblique position between horizontal and vertical lamellæ or wedges; it is used particularly in the case of the Carchariidæ.

Unless otherwise stated, the word "section" in this paper is used in the sense of a transverse section taken midway between the anterior and posterior ends of the centrum, *i.e.*, through the narrowest part of the double cone.

HISTORICAL REVIEW.

The monumental work on the calcification of the centra of the sharks and rays is the monograph by C. HASSE, entitled 'Das Natürliche System der Elasmobranchier auf Grundlage des Baues und der Entwicklung ihrer Wirbelsäule.' In most libraries the monograph is bound as a single volume of 392 pages and 48 plates, but the work was issued as a series of five parts, extending over a period of six years (1879–1885). HASSE had interested himself in the subject before 1879, and the results of his earlier work on fossil vertebræ (**14**) are included in the monograph.

Of the papers published prior to 1879 the most important are those of KÖLLIKER (**19** and **20**) and GOETTE (**13**); an abstract of the still earlier writings by RATHKE, JOH. MULLER, and LEYDIG is given by HASSE in his paper of 1892 (**16**).

Since 1885, the date of the issue of the final part of HASSE's monograph, but little in the way of original investigation has been done upon the vertebræ of sharks and rays, most writers who have had occasion to allude to the subject being content to make excerpts from HASSE's book. This, it may be observed in passing, is a good

illustration of the paralysing effect that a bulky work has upon research in any particular branch of science. The publication of an imposing monograph creates an impression that the particular subject with which it deals is now disposed of; and unless it opens up controversial matters, the monograph chills the enthusiasm and checks the aspirations of those who might otherwise be tempted to write upon the subject. Small papers, on the other hand, have rather the effect of encouraging the production of others upon the same theme, investigators interested in the subject being induced thereby to add their quota to the sum total of existing knowledge.

Among the papers that have been produced since the issue of the final part of the monograph is one by HASSE himself (**16**), papers by KLAATSCH (**18**), a paper by GADOW and ABBOTT (**6**), and a contribution by SCHAUINSLAND, largely a compilation, but partly original, in HERTWIG'S handbook (**30**). While these deal with the development of the vertebræ of sharks and rays rather than with the value that may be attached to the pattern of the calcified areas as a means of classification, it is to be observed that development has an important bearing upon the question, and cannot be disregarded in any discussion of HASSE'S conclusions.

REGAN'S paper of 1906 (**29**), dealing with the classification of the Elasmobranchii, contains numerous references to the calcification of the centra in adult fishes. The criticism of HASSE'S scheme is in the main adverse, but the paper offers little that is constructive in its place; by recommending the retention of HASSE'S terms with new, restricted meanings (**29**, p. 737), the author makes a difficult subject rather more confused than before.

DEVELOPMENT OF THE CENTRA.

The notochord in the course of its development becomes invested by an envelope or sheath, produced by the chordal cells. This envelope in the case of Elasmobranch fishes undergoes differentiation into an external cuticular sheath, the membrana elastica externa, and an inner fibrous sheath, which is considerably thicker.*

On the differentiation of the sclerotomes from the myotomes the skeletogenous tissue applies itself to the sides of the notochordal sheath, but the right and left tracts of skeletogenous tissue are not continuous above and below the notochord. At a later period the arch-cartilages begin to differentiate in the upper and lower parts of the tracts, while the middle part of each tract becomes reduced, and thinned out into a layer of about two cells in thickness.

Skeletogenous cells now invade the fibrous sheath of the notochord through fenestrations in the membrana elastica externa,† particularly from the arch-bases, and

* Good *résumés* of the opinions of various investigators regarding the structure and origin of the layers of the notochordal sheath are given by HASSE (**16**) and SCHAUINSLAND (**30**, p. 395).

† The origin of the cartilage of the notochordal sheath by immigration of skeletogenous cells was first observed by HASSE (**16**, 1892, figs. 10 and 11), although his interpretation of the process is different from that now generally accepted. The invasion through the membrana elastica externa was explained by KLAATSCH (**18**, II, 1893, p. 162, and Plate 7, fig. 8), and the observation has been confirmed by GADOW

produce a thickness of cartilage between the outer membrane (membrana elastica externa) and a structureless layer which is now recognisable as the membrana elastica interna.* The chondrified sheath is not for long continuous and uniform along the notochord, but soon differentiates into short tubes, or rings, of hyaline cartilage set end to end.† The rings thicken and lengthen, and develop into the vertebral bodies or centra, the chordal sheath in the intervening regions passing into a fibro-cartilaginous and ultimately into a fibrous condition, and persisting as the "inter-vertebral ligaments." In the middle of the length of each centrum thus delimited the notochord becomes constricted by the rapidly growing cartilage, but inter-vertebrally the notochord continues to increase in width, as well as in length. In this way there arises a series of typically biconcave or amphicœlous centra.

In *Hexanchus griseus*, *Heptanchus cinereus*, *Echinorhinus spinosus* and *Somniosus microcephalus* the intervertebral portions of the axial column are vastly longer than the vertebral portions, which latter are, except in the caudal regions of the second and fourth of these species, indicated merely by large fibrous septa set transversely to the notochord. In these four species the notochord is to a large extent liquefied in the adult. *Chlamydoselachus anguineus* in some respects resembles *Heptanchus*, but the differentiation of the notochordal sheath into structures that can be recognised as vertebral centra is, on the whole, less advanced (see p. 348).

The neural and hæmal arches of the vertebræ are developed independently of the centra,‡ except in so far as the immigration of cartilage-forming cells into the sheath proceeds to an exaggerated extent, if not entirely, at the arch-bases. The arch-

and ABBOTT (**6**, 1895, p. 164), and SCHAUINSLAND (**30**, 1906, pp. 397 and 407). Whether the cells penetrate between the two layers of the sheath, or pass through the outer membrane and become lodged in the substance of the thickening inner layer, is a matter foreign to the scope of the present investigation; for a discussion of this point the reader is referred to SCHAUINSLAND'S summary (**30**, p. 397).

* This early invasion of the notochordal sheath renders the development of the centra of the Elasmobranch fishes profoundly different from that in other Vertebrates, and the fishes are consequently unsuited for drawing general deductions as to the morphology of the parts of vertebræ. An invasion of cartilage-forming cells into the chordal sheath occurs in Holocephali and Dipnoi, but not to the same extent as in the Elasmobranchii, and true centra are not formed. In other Vertebrates, the chordal sheath remains intact, except in so far as it is absorbed and replaced; and the "bodies" of the vertebræ are formed external to it. In Amphibia and Amniota the "bodies" appear to be formed almost entirely from the tissue of the neural and hæmal arches (GADOW, 'Phil. Trans.,' B, vol. 187, pp. 1–57, 1897), but in fishes with bony vertebræ (bony Ganoids and Teleosteans) there are independent contributions in the form of "bone-belts," deposits of bone in neutral fibrous tissue, adjacent to, but not derived from, the arch-bases (see p. 335 of the present paper).

† CARTIER, **2**, Plate 4, figs. 1–2; RABL **28**, Plate 6, figs. 9–11; KLAATSCH, **18**, II, p. 172, text-figs. 3–4; SCHAUINSLAND, **30**, p. 402, text-fig. 206.

‡ GADOW and ABBOTT (**6**, p. 218) write: "The formation of chordacentra being independent of the arcualia explains how and why the number of 'centra' does not necessarily agree either with that of the arcualia or with that of the trunk-segments, *e.g.*, *Hexanchus*, and tail of most other Elasmobranchs." See also **6**, p. 194.

cartilages are morphologically external to the notochordal sheath, but owing to the fenestration, or to the total disappearance of the membrana elastica externa at the regions of apposition of the dorsal and ventral arches, the arch-cartilage becomes continuous with the cartilage of the sheath, and the limit between the two is no longer discernible.

The neural arches are complete, the principal components being basidorsal cartilages and interdorsal cartilages (intercalary plates); sometimes there are present small unpaired supradorsals and supraiinterdorsals as well. Supradorsals are wanting in the Lamnidæ, and in some parts of the vertebral column the right and left basidorsals and the right and left interdorsals are confluent above the spinal cord. In the Orectolobidæ there is a tendency for the supradorsal cartilages to be small and disposed in a double series, right and left, not necessarily in pairs, however, for in some parts of the vertebral column the right and left elements alternate.

The hæmal arches are complete in the tail, and are constituted by the basiventral cartilages; interventral cartilages (intercalaries) are sometimes present, but with less regularity than the dorsal intercalaries. In the vertebræ that are situated anteriorly to the cloaca the hæmal arch is incomplete, and the basiventral cartilages are commonly termed "transverse processes"; they are continued at their outer extremities into the ribs.*

According to Gadow and Abbott (**6**, p. 171, No. 2; p. 173, No. 7; p. 189; and text-figure on p. 188) the intercalary cartilages arise by a severance of processes from sclerotomes whose major portions become the basalia. A dorsal pyramid of skeletogenous cells spreads downward at the side of the notochord, and its lower end becomes an interventral cartilage, while the more bulky upper portion develops into the basidorsal cartilage; similarly the interdorsal and basiventral cartilages are the upper and lower products of a tract of formative cells which, originating as a ventrolateral cluster, has spread upward at the side of the notochord.

The basidorsal cartilages are, with certain notable exceptions (Hexanchidæ, and the region of transition from the monospondylous trunk vertebræ to the diplospondylous caudal vertebræ in most Elasmobranchs), commonly set over the middle of the length of the centrum, whereas the interdorsals are set over the intervertebral ligaments. The ventral root of each spinal nerve issues behind the basidorsal or passes through it, and the dorsal root passes out either behind or through the interdorsal or intercalary plate.

The centra and the arches of the vertebræ are fairly rigid; movements of the vertebral column as a whole are permitted solely by the flexibility of the intervertebral ligaments and the tracts of white fibrous tissue that occur between the several plates of cartilage that constitute the neural and hæmal arches. There are

* The vexed question whether the ribs in Elasmobranch fishes correspond with the ribs of the higher Vertebrates is discussed by Schauinsland, **30**, pp. 423–427.

no zygapophysial articulations of the neural arches such as occur in Teleostean fishes and bony Ganoids.

The cartilaginous layer of the notochordal sheath is early differentiated into three zones, the inner, middle and other zones of GOETTE, HASSE and others. The middle zone, composed of fibro-cartilage, soon undergoes calcification (except in the few species mentioned in a previous paragraph), and becomes the well-known "double cone" or dice-box of the vertebræ of sharks and rays (fig. 2, p. 321). The inner zone is usually composed of hyaline cartilage, and in the adult occurs principally in the region where the double-cone is narrowest (fig. 7, B, p. 352); it here constricts the notochord, sometimes to obliteration. Towards the anterior and posterior ends of the centrum it passes over into fibro-cartilage and fibrous tissue.

In some cases, where the obliteration of the notochord in the middle of the centrum is complete, or nearly complete, in the adult, a fibrous septum of considerable extent separates the persistent remnants of notochordal substance, as in *Hexanchus* and *Echinorhinus* (fig. 23, p. 373). In other cases the notochord is divided by a calcareous partition of small extent (*Scyliorhinus stellaris*, fig. 16, B, p. 363; *Cestracion philippi* and *Raia marginata*, fig. 4, p. 340). In less aberrant cases (Squalinæ) there is a persistent thin layer of cartilage (inner zone) immediately internal to the double-cone calcification, at the part where it is narrowest, and within this is a tract of persistent notochordal jelly, which shows a tendency to be tougher than usual, and to develop fibres longitudinally disposed. Between these extreme cases are various intermediate forms, in which the inner zone is present in the form of an incomplete septum, either of hyaline cartilage or of fibrous tissue, showing a concentric arrangement of the fibres; and running antero-posteriorly through the middle of this is a fine thread of notochordal substance, not jelly-like, but of a fibrous character, commonly termed the "funicle." In the hindermost caudal vertebræ of many forms (*Scyliorhinus stellaris*, *Raia marginata*), there is a long thin plug of hyaline cartilage, sometimes, but not always, perforated by a funicle.

The outer zone, composed at first of hyaline cartilage, grows in thickness, and in some forms (*Cestracion*, *Squatina*, *Pristiurus*, *Squalus*) constitutes a large proportion of the substance of the centrum. It may in the adult undergo partial calcification, by the development of an investing layer ("Belegschicht" of HASSE), immediately external to the double cone, and in intimate contact with it (fig. 22, p. 372). Or there may be developed in the outer-zone cartilage a number of calcareous lamellæ, longitudinally disposed. These lamellæ may radiate outward from the primary double cone, so that a transverse section of the centrum exhibits a star-like pattern (*Cestracion*, fig. 4, A, p. 340); or the calcified lamellæ may be in the form of concentric, longitudinally set tubes, so that a transverse section exhibits a series of concentric rings (*Squatina*, fig. 25, p. 377). Such calcified lamellæ as these are usually termed secondary lamellæ, since they are developed later than the primary double cone.

The calcification, both primary and secondary, consists of a deposition of lime-salts in the intercellular matrix of the cartilage; it is not a redeposition of calcified tissue following upon an absorption of the original calcified matrix, such as one understands to be the distinguishing feature of true bone. The cells of the original cartilage persist as the cells of the ultimate calcified tissue, and are not immigrant cells like the cells of typical cartilage-bone.*

The double cone maintains its independence from the secondary calcification, and its outer limit can usually be distinguished by structural features even in cases where there is a uniform investing deposit of outer-zone calcification immediately external to it (fig. 22, p. 372).

The external surface of the centrum is rarely (*e.g.*, *Echinorhinus*) free from calcification. In the Squalinæ the superficial calcification usually takes the form of a thin layer, that differs from the layers found on the inner and outer surfaces of the arches in being a continuous crust, instead of a mosaic of tesseræ; but in cases where there is evidence of the neural arch having united with the hæmal arch external to the notochordal sheath, the layer of tesseræ may be continuous from arch to arch (*e.g.*, *Narcine*, HASSE, **15**, C, Plate 23, fig. 20). In other cases there may be calcified deposits so thick as to present the form of four calcified wedges, one dorsal, one ventral, and two lateral, with their bases external and their edges directed towards the vertebral axis (*e.g.*, *Mustelus*, fig. 8, A, p. 354).

These wedges are produced, as was first shown by KÖLLIKER in 1860 (**19**), by the activity of the superficial perichondrium that occurs external to the membrana elastica externa of the sheath in four tracts, namely, between the paired components of the neural arch, between those of the hæmal arch, and between the basidorsal and basiventral on each side of the notochordal sheath.

While these "Knochenzapfen" (KÖLLIKER, **19**), "Verbindungsstücke der Bögen" (GOETTE, **13**), or "Periostale Keile" (HASSE, **15**) are a characteristic feature of the Carcharidæ, they are also present, though in a less completely calcified form, in Lamnidæ, Odontaspididæ, Orectolobidæ, and Scyliorhinidæ. Whether fully or incompletely calcified, the structures in question are centrifugal in their mode of growth, the inner parts being the oldest and the outermost layers the most recently added.

The wedges of perichondrial origin do not find a place in the series of terms introduced by GADOW and ABBOTT in 1895 for "the cartilaginous pieces which form one Skleromere or Vertebra" (**6**, p. 170). The arch-cartilages (arcualia) of their terminology include the basalia (basidorsalia and basiventralia) and interbasalia (interdorsalia and interventralia), together with supradorsalia, etc. It is only the

* Among those authors who consider that "bone" occurs in sharks and rays may be mentioned STARK ('Trans. Roy. Soc., Edinburgh,' xv, 1844, pp. 646 and 654), and GOETTE (**13**); opposed to this view are KÖLLIKER (**20**), GEGENBAUR (**9**), HASSE (**15**, B) and others. A discussion of the question took place in the pages of 'Nature' in 1898 (vol. 58, pp. 26 and 200).

basalia that are of interest in the present connection, for the others do not form part of the definitive centrum.

The four tracts of skeletogenous tissue and their ultimate products, situated above, below, and at the sides of the chordal sheath, between the four basalia as seen in a transverse section, are tracts of more importance than is conveyed by the term "Verbindungsstücke" of GOETTE and SCHAUINSLAND; they are in many cases determinate constituents of the centrum, not mere joining-up pieces. For the designation of these I propose the term *intermedialia*, a term to be employed not only for the sharply differentiated calcified wedges of the Carchariidæ, and their equivalents in the Lamnidæ, etc., but also to be applied in other cases, such as *Squalus* (fig. 22, B, *p*, p. 372), in which the precise limits of the tracts of perichondrial origin are in the adult difficult to distinguish, although a study of the embryonic vertebræ shows that they are in reality developed separately from the basalia. That the intermedial tracts in the British species of *Scyliorhinus* (*Scyliorhinus canicula* and *Scyliorhinus stellaris*) are reduced "periostale Keile," such as occur in the Carchariidæ and Lamnidæ, is clear from a comparison of the British with the more southern species, *Scyliorhinus marmoratus* and *Scyliorhinus maculatus* (*cf.* fig. 16, p. 363, and fig. 15; and see HASSE, **15**, D, Plate 34, figs. 19, 21, and Plate 33, figs. 2, 5).

It is to be noted that REGAN, one of the more recent critics of HASSE'S "system," while not regarding the great calcified wedges of the Carchariidæ as originating from arch-tissue, does not discuss the question of their perichondrial origin. He writes (**29**, p. 736, footnote): "This extension of the neural and hæmal plates round the chordal sheath appears to be often inversely proportional to the secondary calcification of the centra. In nearly all the Galeoidei the calcifications extend throughout the centrum, and the neural and hæmal arches do not extend downwards or upwards, but in *Pristiurus*, where the secondary calcification has disappeared, they meet round the centra." It may here be observed that the series *Scyliorhinus marmoratus*, *Scyliorhinus canicula*, *Pristiurus melanostomus* (fig. 15, B, p. 363; fig. 16, A and C) points to the bulk of the cartilage of the centrum in the last of the three being of sheath origin, and not due to union of the basidorsal and basiventral cartilages, for in the second of the three species the vestiges of the great wedges are recognisable, and they develop late and at a much greater distance from the axis of the centrum than in the first. The clue is given by the section of *Scyliorhinus marmoratus* through the caudal fin (fig. 15, C); if the lateral calcifications were as little developed as the upper vertical, and flatter, the appearance would be much the same as in a caudal centrum of *Scyliorhinus canicula*, but there is no question in *Scyliorhinus marmoratus* of a union of basidorsal and basiventral cartilages *internal* to the lateral calcification, for the membrana elastica externa is contiguous with the intermediale. It is true that in *Scyliorhinus canicula* and *Pristiurus melanostomus* there is, between the membrana elastica externa and the calcified intermediale, a narrow tract of cartilage whose origin it is difficult to trace, but it is evident it is not to this, but to the great mass of

cartilage marked *o z* in fig. 16, A and C, that REGAN is referring when he speaks of the "meeting of the neural and hæmal arches."

It is possible that had KÖLLIKER seen the vertebræ of *Scyliorhinus marmoratus* or *maculatus*, he might not have written "Knorpelrinde der Bogen vollständig" for *Scyllium* (probably *canicula* or *stellare*), seeing that for the Carchariidæ and Lamnidæ he wrote "Knorpelige Bogen nicht verschmolzen." In both of his papers (**19**, pp. 210 and 231, and **20**, p. 74) he regards the four thick calcifications of *Scyliorhinus* as "Periostablagerungen" *on the outside* of the fused arches.

Returning to the characteristics of the more typical intermedialia, it is to be noted that whereas the basalia, so soon as they can be distinguished in ontogeny, are composed of embryonic hyaline cartilage, and after the embryonic stages grow by a kind of uniform expansion, the intermedialia develop late, and are produced by skeletogenous connective tissue, which generates cartilage on its inner face, and continues to generate layer after layer. The corresponding "intermedialia" of *Amia* and *Esox* calcify directly, without any intermediate condition of cartilage; they arise that is to say, as membrane-bone (SCHAUINSLAND, **30**, p. 444, fig. 243; p. 469, fig. 263). In *Ceratodus* and *Acipenser* the intermedial tracts remain in the form of indifferent, non-skeletogenous tissue (**30**, p. 483, fig. 274A; p. 432, fig. 228).

It is unfortunately necessary to be arbitrary in instituting a terminology for morphological entities that are differentiated out of the same original substance. The practice has been adopted by previous writers in their distinction of the arch-bases of Elasmobranch fishes from the sheath-cartilage, although the latter is produced from the former by a process of invasion of cartilage-forming cells through gaps in the membrana elastica externa. What is meant in their writings is that the term arch-bases (basalia) shall designate tracts of arch-cartilage external to the membrana elastica externa, while sheath-cartilage is cartilage situated internal to the membrane, or, where in adults the membrane is no longer distinguishable, cartilage which has been derived from that cartilaginous layer which in the embryo was situated internal to the membrana elastica externa.

Similarly with regard to the intermedialia; a distinction between basale and intermediale visible in the embryo may be lost in the adult, but it is convenient, nevertheless, to retain the distinction in adults, if only to dispel the idea of an arch-fusion external to the notochordal sheath. Arch-fusion certainly does occur in some Elasmobranchs, but it is in other cases possible to conceive that the basidorsal cartilage may in the adult be separated from the basiventral cartilage by an intermedial uncalcified cartilage which has originated *in situ*, and has not arisen by a creeping or spreading of the basal cartilages towards one another.

Although for the sake of brevity of expression it is stated above that the growth of the intermediale is due to the perichondrium, it is only in the initial stage that the perichondrial connective tissue itself is active. After that stage there exists, immediately internal to the part that persists as the perichondrium, a zone of small-

celled, growing cartilage which is responsible for the expansion of the intermediale in a radial direction. This genetic zone or layer is three, four or five cells in thickness; the innermost cells gradually enlarge, and become more separated by cartilage-matrix, and thus add to the bulk of existing cartilage; the outer cells continue to multiply. Parts of the intermedialia that are destined to be calcified may undergo calcification almost immediately, so that in such cases the calcified lamina or wedge reaches nearly to the surface, being separated from the perichondrium proper by the thin growing zone only.

The precise mode of origin of the genetic layer of the intermediale from the perichondrium is in a few cases liable to misinterpretation, in consequence of the fact that the differentiation begins at the edges of the basalia, and does not commence simultaneously over the whole area. In a young *Galeus canis*, 230 mm. in length, the section of a vertebra between the pelvic and second dorsal fins (fig. 7, C, p. 352) shows the upper, lower and lateral parts of the membrana elastica externa still external, and covered by perichondrial connective tissue only. The basidorsal and basiventral cartilages thin out almost to nothing over the upper and lower parts of the lateral tracts, and it might be argued that in the later stages of development these cartilages spread towards one another, between the membrana elastica externa and the connective tissue, and ultimately meet. But careful examination of the slightly different stages that can be seen in the vertebræ of the fish in question—the development, for instance, reaches slightly different stages in consecutive vertebræ, and even on the two sides of the same vertebra—shows that it is the "cellular activity" that spreads, not the "cellular tissue." The cells that will, by subsequent division, produce the substance of the intermediale are differentiated from the connective tissue at the edges of the basalia, and are not immigrants from the basalia themselves. At the same time that these external changes are taking place the activity of the cartilage immediately internal to the membrana elastica externa is increased, and the inner and outer tissues soon become continuous.

In the vertebræ of a late embryo of *Mustelus*, of a length of 286 mm. (fig. 7, A, p. 352), exhibiting a slightly later phase of development than is shown in fig. C by the *Galeus* embryo, the sharp delimitation of the edges of the developing intermedialia may be to some extent taken as evidence of their independence from the basal cartilages. A later stage still would be that illustrated by the section of a young *Carcharias* vertebra (fig. D), and this leads on to the condition seen in the adult *Mustelus* (fig. 8, A, p. 354).

The developmental history of the intermediale of the Carchariidæ as set out above would no doubt have been more convincing if the successive stages had been worked out in embryos, young and adults of one and the same species, but such a series was not accessible. The facts as here elucidated are, however, in my opinion, such as will stand the test should a large series of *Mustelus*, *Galeus*, *Carcharias* or *Sphyrna* at some later time become available for investigation.

Another series illustrating the development of the intermedial components of the centrum is that shown in fig. 17, p. 365. *Chiloscyllium* was obtained in three stages of development, namely, an embryo of a length of 120 mm., one of a length of 240 mm., and an adult. The series of sections of caudal vertebræ is interesting as showing how each intermediale begins its development as a double structure (p' and p'' in fig. E). Each component enlarges, particularly in a radial direction (p' and p'' in fig. F), and the sheath-cartilage grows out horizontally between them and pushes the membrana elastica externa before it. After the stage of development figured at F, the perichondrium between p' and p'' becomes active and produces a large mass of cartilage, which does not undergo calcification (fig. D, p). The sheath-cartilage now presents the appearance of bulging out into the intermediale between the two radiating calcified lamellæ. In the trunk vertebræ the bulging is much more pronounced, and the middle part of the perichondrium of the upper median intermediale, as also that of the lower median, fails to become active as a cartilage-producer, and the membrana elastica externa reaches the surface of the centrum (figs. A and B).

Whereas in *Chiloscyllium* the sheath-cartilage continues to grow for a time in the vertical and lateral directions, and projects into the intermedialia, it is to be noted that in the Carchariidæ the sheath-cartilage grows out in the diagonal planes, and projects into the arch-bases. A study of the Carchariid material available goes to show that the diagonal calcified lamellæ are centrifugally-growing calcifications of the sheath-cartilage (fig. 8, p. 354), and although in late stages of development the most peripheral parts of the membrana elastica externa, at the extremities of the outgrowths of sheath-cartilage, are no longer to be recognised, it may be concluded, on the whole, that the diagonal calcified lamellæ belong to the sheath-cartilage. Nevertheless, one is not justified in making too arbitrary statements about the matter, for when calcification begins in cartilage of a certain origin, and has proceeded for a time in that cartilage, it may be continued later into cartilage of a different origin. Instances of this kind were observed in the vertebræ of one specimen of *Mustelus*. As shown in the upper right-hand diagonal tract of fig. 8, A, p. 354, the calcification of the diagonal ray, begun in the sheath-cartilage, has continued into the basidorsal cartilage. It is as though, in the course of the radial growth of the calcified lamella, the membrana elastica externa became caught or entangled in the calcifying tract, instead of being pushed outward beyond the area that is undergoing calcification. This aspect of the case seems to be justified from the convex appearance of the membrana elastica externa on the two sides of the calcified lamina; the sheath-cartilage here tends to bulge outward, but the middle part of the membrana elastica externa is fixed in the calcified lamina, and cannot follow the rest of the membrane as it is pushed radially outward.

In the Batoid fishes, the interpretation of the calcified structures in the centrum is rendered difficult, in consequence of some of the radiating lamellæ (*e.g.*, the vertical and horizontal rays in the caudal vertebræ) being of double origin. The

inner parts, nearest the double cone, are derived from the outer zone of the sheath-cartilage, whereas the more peripheral parts are of perichondrial origin (*Rhinobatus* and *Dasybatis*, fig. 30, p. 383; and fig. 35, p. 391). Owing to the early disappearance of the membrana elastica externa in these Batoids, it is not possible to indicate any sharp delimitation between the two component parts. Although the lateral rays reach the surface in the middle portion of the tail, they do not reach the surface in the trunk region, where the basiventral cartilages are set so high up the side of the notochordal sheath as to unite with the basidorsals (fig. 32, A, p. 387); neither do they reach the surface in the vertebræ from the hinder part of the tail (fig. 30, D and E, p. 383). In some cases the internal and external components are distinct, and are separated by a tract of cartilage (fig. 32, D and E, p. 387); but, owing to the absence of any relics of the membrana elastica externa, it is not possible to say with any degree of conviction that the inner portion is developed solely in the sheath-cartilage, and that the outer part is solely of perichondrial origin.

By a happy chance, embryos of *Rhinobatus*, *Trygonorhina*, and *Myliobatis* were obtained at that stage of vertebral development when the secondary calcification, uniformly enlarging within the outer-zone cartilage, has just arrived so near the surface of the centrum that the perichondrium is coming into play as an active layer. In the caudal vertebræ there are four such perichondrial tracts (fig. 28, C, p. 382), and the conclusion to be drawn is that subsequent growth of the vertical and horizontal lamellæ is due to the perichondrial reinforcements, whereas the diagonal rays (fig. 30, B, p. 383; and fig. 28, C, *d*, p. 382), being independent of perichondrial activity, rather resemble the diagonal rays of the Carchariidæ. They are probably confined to the outer-zone cartilage, but there is no evidence to prove that they do not grow into the basal parts of the arch-cartilages. The membrana elastica externa being no longer recognisable, the limits of the sheath-cartilage cannot be definitely determined.

In *Torpedo* the external component seems to be wanting (fig. 26, B, p. 379), since the calcified rays do not quite reach the surface. There can be no doubt that the basal parts of the radiating lamellæ are of sheath-origin, for the upper and lower parts of the membrana elastica externa are still recognisable at a time when the secondary calcification around the double cone has already taken on a radiating appearance (fig. 27, p. 380). But the fact that the calcified rays of the adult do not actually reach the surface cannot be taken as proof that their outermost parts are not of perichondrial origin, for several instances are known in which there is delay in the process of calcification of perichondrially produced cartilage. While the cartilage that occurs between the outer edge of the calcified ray and the surface of the centrum in the adult *Torpedo* is a small-celled growing cartilage, there is not the same evidence as there is in the horizontal and vertical rays of caudal vertebræ of *Rhinobatus* and *Trygonorhina* that this tract is of perichondrial origin. In *Narcine*, another genus of the Torpedinidæ, the evidence of sections from a young specimen,

220 mm. long and 105 mm. broad, is in favour of the perichondrial origin of the outer parts of some of the rays, there being well-marked fibres in the cartilage, extending from the superficial connective tissue for a certain distance inward into the calcified rays.

In *Pristis* and *Rhynchobatus* there is a well-marked growing zone of small-celled, delicate cartilage, situated immediately external to the dense and uniform secondary calcification characteristic of these fishes (fig. 31, B, *g*, p. 385). In an embryo of *Pristis pectinatus*, of a total length of 265 mm., and in an embryo of *Rhynchobatus djeddensis*, of a length of 102 mm. (fig. 28, A, p. 382), the remains of the membrana elastica externa are still visible, and these are situated external to the growing zone. When the membrane disappears, as it does soon after the stage of development indicated, the growing zone continues its activity, and adds to the calcified mass already existing. There is thus proof that the growing zone is at first within the sheath-cartilage, and there is nothing to show that the later contributions to the calcified mass are of different origin from the former.

In *Cestracion* and *Squatina* also there may be recognised a growing zone, which in transverse sections presents itself as a ring of small-celled cartilage, situated a short distance external to the calcified rays of the former and the last-formed calcified ring of the latter. In *Cestracion* (fig. 21, A, p. 370), the remains of the membrana elastica externa, visible in the adult, are situated a short distance external to the growing zone, which is thus seen to belong to the chordal sheath. In *Squatina* the membrane is still visible in a ripe embryo (fig. 13, B, p. 360), and its position external to the growing zone at a time when there are four calcified rings present suggests that the later calcified rings of the adult (fig. 25, p. 377), like the early rings of the embryo, are all of sheath-origin.

There seems to be no special region of growth of the cartilage of the centrum in the Squalinæ, nor in such members of the Scyliorhinidæ as *Scyliorhinus stellaris* and *Pristiurus melanostomus*. The evidence in these species points to a uniform expansion of the cartilage. In the Carchariidæ, the positions taken up by the remnants of the membrana elastica externa go to show, as is pointed out above, that there is an out-pushing of the cartilage along the diagonal lines, in marked contrast with the failure of the sheath-cartilage to extend along the vertical and horizontal radii, where growth is provided for by the superficial perichondrium (fig. 8, p. 354), but, in some other Galeoidei, the out-pushing of the sheath-cartilage is, at all events for a time, towards the perichondrial tracts, and not along the diagonal lines (fig. 17, B, p. 365).

The arch-cartilages as a rule grow in thickness by a general expansion, as is evident from the even spacing of the cartilage cells in such of the middle parts as are uncalcified, and from the fact that the calcified tesseræ of the arches, both internal and external, develop in a nearly superficial position, and retain this relative position throughout life. Even in those cases in which the edge of the layer of

tesseræ is no longer superficial (*e.g.*, *Galeus;* hindermost caudal vertebræ of *Alopias*, fig. 10, p. 357), the result has been brought about by an overgrowth of the outer parts of the wedges of perichondrial origin, and not by superficial additions to the arch-bases themselves.

Chorda-centra and Arco-centra.

Although Gadow and Abbott (6) distinguish the centra of Elasmobranch fishes as chorda-centra, developed from the cartilage of the invaded notochordal sheath, whereas those of Amphibia and Amniota are arco-centra, developed from the component parts of the neural and hæmal arches, it is to be noted that it is only in some Elasmobranchs (*e.g.*, *Cestracion*, *Squatina*, *Pristiurus*) that the substance of the "body" or centrum is to any great extent derived from sheath-cartilage. In a large proportion of cases there are substantial contributions from the arches (*e.g.*, diagonal tracts of cartilage in Lamnidæ, etc., lateral parts of the centra of trunk vertebræ of Batoid fishes), and from intermedial tracts of superficial perichondrium between the arch-bases (*e.g.*, vertical and horizontal wedges of Carchariidæ and Lamnidæ).

As an addition to the literature of the "Neugliederung" of the vertebral column, the paper by Gadow and Abbott is of considerable value, since these authors show that the phenomenon is not a secondary one, due to the tracts of skeletogenous tissue that arise from the protovertebræ splitting and recombining to form the new segments, but is primary, and arises from the building up of the vertebræ by the association of skeletogenous cells formed from the posterior part of one body-segment with those from the anterior part of the next (6, pp. 187–189); but, where one may be disposed to differ from the authors, is in their disregard of the generative capacity of the middle portions of the dorso-ventrally extended pyramids of the sclerotomes.

Their views are summarised in the following paragraph (6, p. 189): "Each ventral pyramid"—*i.e.*, ventral portion of the sclerotome—"extends with its apex above the chorda, and founds there (separated from the ventral mass by the subsequent rapid growth of the chorda and its sheath) a cluster of cells, which remains henceforth behind the basal mass of the dorsal pyramid; this latter, through its downgrowing apex, founds a colony of cells, but below the chorda and in front of the basal ventral mass. Thus are produced the basalia and interbasalia. Each colony of cells retains its potentiality of developing into a separate independent piece of cartilage."

The cell-clusters that become the basidorsal and interdorsal cartilages are stated to undergo separation from those that develop into the interventral and basiventral cartilages by the rapid growth of the chorda and its sheath. This appears to be in accordance with the facts, for transverse sections through embryos of the appropriate stage of development show, at the sides of the notochord, a thin layer of not more

than two or three cells; but, when the authors proceed to affirm that the dorsal and ventral parts are "connected only by the indifferent connective tissue of the membrana reuniens, but not by cartilage-forming cells," a protest may be lodged, for, while at this particular epoch of development the connective tissue is "indifferent," it really does in many cases possess the capacity for forming cartilage. The capacity is not evinced until the arch-cartilages are well differentiated.

It is unfortunate that GADOW and ABBOTT confined their studies to early stages of development, before the intermedial tracts become active as skeleton-producers, and that they limited their observations, on Elasmobranch fishes, to *Acanthias vulgaris*, *Centrophorus granulosus*, and *Scyllium catulus*,* for these are not well suited for a study of the products of the intermedial tracts. Had they included in their series some fairly late embryos of *Lamna*, *Alopias*, *Carcharias*, *Sphyrna*, or *Scyliorhinus marmoratus*, their decisions might have been different.

The centra of the Teleostean fishes and the bony Ganoids are, according to GADOW and ABBOTT, arco-centra, the bulk of the substance of the definitive centrum in these fishes being developed from the neural and hæmal arches; but if, as is claimed in the present paper, the great wedges of the Carchariid sharks are "intermedialia," *i.e.*, distinct entities developed from skeletogenous connective tissue occurring in four tracts between the edges of the arch-bases (p. 328), the same mode of origin must be accorded to the bulk of the bony substance of the centrum in the Teleosteans and Ganoids. GADOW and ABBOTT, indeed, admit that in the Trout very little arch-cartilage grows round the chordal sheath, and that "most of the material necessary for the formation of the centrum is indifferent membrana reuniens, and ossifies directly" (**6**, p. 216; apparently after SCHEEL, 'Morph. Jahrb.,' xx, 1, 1893).

Again, as regards the origin of the "bone-belts" of *Amia*, GADOW and ABBOTT are not altogether consistent, for, while on p. 207 they state that the spaces in which the bone-belts arise "are filled with indifferent connective tissue and ossify early"—a statement which is fully in accord with the later observations of SCHAUINSLAND (**30**)—yet on p. 208 they say that the centrum is "a compound structure, produced entirely by the arcualia."

On p. 202 they write: "Outside this elastica (externa) follows a thick zone of loose connective tissue which forms a layer of bone on its inner surface, and in this zone of connective tissue cartilage cells, from the basal portions of the arcualia, grow round the chordal sheath preparatory to the formation of the central discs." This spreading of cartilage-cells from the arch-bases is not convincingly shown in their text-figures of the sections studied (p. 204), and is not confirmed by SCHAUINSLAND, who, reviewing the work of SCHMIDT (1892), HAY (1895), and GOETTE (1897), and submitting original observations of his own, regards the bony masses of the centrum as not being preformed in cartilage (**30**, pp. 437–449). It is true that, in

* These are the names as given by GADOW and ABBOTT; the first and third are in the present paper termed *Squalus acanthias* and *Scyliorhinus stellaris*.

the foremost two or three vertebræ, immediately behind the skull, the hæmal arches are set high, and nearly touch the neural arches (fig. 238, p. 440), and they are partly divided into a rib-carrying transverse process and a more ventrally placed mass of cartilage, which in the vertebræ of the middle part of the trunk is segregated as the cartilaginous core of the aortic support (fig. 239, *h*, p. 440); also, at the extreme posterior end of the tail there is a continuous cartilaginous sheath (p. 439); but, as regards the vertebræ of *Amia* generally, SCHAUINSLAND writes: "Auch darin stimmt *Amia* mit den Stören überein, dass die unteren und oberen Bögen durch einen weiten Zwischenraum getrennt und nicht durch Knorpel, sondern allein durch Bindegewebe miteinander verbunden sind" (p. 439; fig. 235, p. 436; see also fig. 243, p. 444, of a later stage, after ossification has begun).

In addition to "bone-belts," GADOW and ABBOTT recognise "cartilage-belts," which, although interpreted by them as arising from pyramids or clusters of skeletogenous cells growing out from the arcualia, may with equal propriety be regarded as autogenous structures, since, in an explanatory footnote on p. 203, they write: "In our 56-mm. *Amia*, the arcualia consist already of hyaline cartilage, with a definite boundary line against the pyramidal or crescent-shaped cell masses. In reality, both the arcualia and these masses are the offspring of the same matrix, namely, of the dorsal and ventral ends of the sklerotomes. Probably the arcualia turn first into cartilage, while the centrum-forming masses grow out later, and still later turn into cartilage, repeating the course of phylogenesis."

Even in the extinct Amioids, there is nothing to preclude the view that certain component parts of the "bodies" of the vertebræ are developed independently of the arcualia. In *Eurycormus* the hæmal arches are attached to the hypocentra, and in the caudal region the neural arches also are attached to the hypocentra, although in the trunk they merely alternate with the pleurocentra, owing to the fact that the hypocentra do not extend sufficiently upward to meet the neural arches. But there is nothing to suggest that the hypocentra and pleurocentra are developed as *outgrowths* from arch-tissue.

In *Osteorhachis* the neural arches alternate with the pleurocentra, and are separate from them. While it is possible, even probable, that there are a pair of interdorsal elements entering into the composition of each pleurocentrum, these elements do not necessarily constitute more than a small portion of it. The fact that the pleurocentrum is not in two parts, right and left, shows that it is not in a primitive condition. If it is composed of interdorsalia only, one would expect to find it a paired structure; if it consists of two interdorsals and a semicircular structure developed from a half-ring of skeletogenous tissue independently of them, the primitive condition would exhibit the triple origin; if, on the other hand, it consists of the half-ring only, then the argument as to its origin from arch-cartilage falls through. As regards the hypocentrum, although the projections that carry the ribs are almost without a doubt basiventral elements, it does not follow that the

hypocentrum is solely a product of the basiventrals. There is always the possibility that both the hypocentra and the pleurocentra are preformed, in part at least, as "bone-belts" from tracts of the general skeletogenous tissue surrounding the notochordal sheath, tracts that are in themselves as independent in origin as the formative tracts destined to grow into the arcualia. The condition found in *Caturus*, in which there is evidence of the paired origin of the hypocentrum, should throw some light on the subject.

On the whole, therefore, the rigid distinction drawn by GADOW and ABBOTT (**6**, p. 190) between chorda-centra and arco-centra in fishes breaks down, for on closer examination it appears that there is not a great difference between *Lamna* and *Alopias*, on the one hand, and *Amia* and *Esox* on the other. In both series the arch-cartilages dip in almost to the middle, in both series the major portion of the remainder of the centrum is formed, not from arch-tissue, nor from chordal sheath, but from superficial skeletogenous tissue between the arch-bases. It is true that in *Lamna* and *Alopias* some part of the centrum is formed out of the chondrified notochordal sheath, whereas in *Amia* and *Esox* the chordal sheath can be disregarded as a component of the adult centrum, but the differences between the two kinds of centra are by no means so pronounced as GADOW and ABBOTT have claimed.

GENERAL ACCOUNT, A CRITICISM OF HASSE'S "SYSTEM."

Introduction.

HASSE'S views regarding the calcification of the centra of the vertebræ of Elasmobranch fishes are expressed in brief in the sentence: "Es können sich entweder um den einfachen, centralen Doppelkegel (Cyclospondylie) in der Aussenzone concentrische Verkalkungsschichten ablagern (Tectospondylie), oder es entwickeln sich in der Umgebung desselben, in der Aussenzone Kalkstrahlen (Asterospondylie)" (**15**, A, p. 29). Except in this solitary instance, HASSE does not apply the three terms to vertebræ, but to groups of fishes, such as "Plagiostomi tectospondyli" (see first footnote on p. 339 of the present paper), but it has become the common practice for writers to speak of cyclospondylous, tectospondylous, and asterospondylous vertebræ.

The three diagrammatic figures that HASSE gives to illustrate cyclospondyly, tectospondyly, and asterospondyly appear repeatedly in text-books and similar works,* and they are here reproduced once more, for purposes of criticism (fig. 3, p. 338).

Opinions differ as to the value of HASSE'S distinctions, but the general impression seems to be gaining ground that, although the differences in the patterns of the

* *E.g.*, BRIDGE, T. W., 'Cambridge Natural History,' vol. 7, London, 1904, p. 198, fig. 113; SEDGWICK, A., 'Text-Book of Zoology,' vol. 2, London, 1905, p. 125, fig. 69; GOODRICH, E S., E. Ray Lankester's 'Treatise on Zoology,' Part IX, fasc. 1, London, 1909, p. 137, fig. 95; KERR, J. G., 'Encyclopædia Britannica,' ed. xi, vol. 24, 1911, Article "Selachians," p. 594; ZITTEL, K., 'Grundzüge der Paläontologie,' Abth. ii, "Vertebrata," Aufl. iii, 1918, p. 45.

calcification of the centra as seen in transverse section are very striking and may prove to be of some taxonomic value, HASSE did not succeed in expressing and co-ordinating the facts in a scheme that adequately meets the requirements of the case. (See Views of Later Authors, p. 344.)

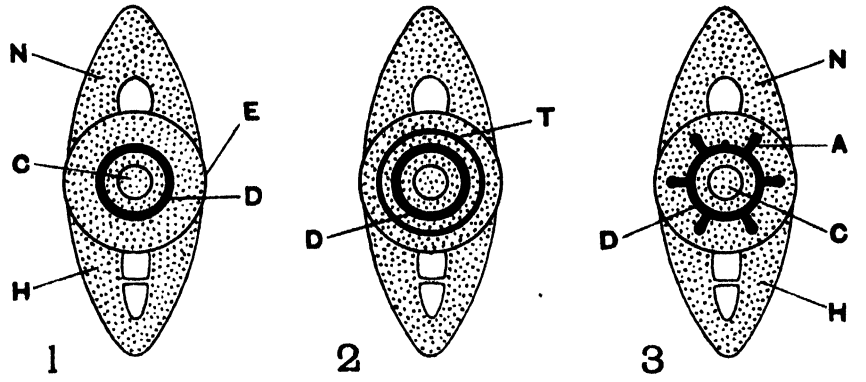


FIG. 3.—HASSE'S diagrams of cyclospondylous (1), tectospondylous (2), and asterospondylous (3) vertebræ, representing transverse sections of anterior caudal vertebræ of the three types, cut midway between the anterior and posterior ends of the centrum.

A, radiating lamellæ characteristic of asterospondylous vertebræ; C, remains of the notochord; D, primary double-cone calcification of the middle-zone cartilage; E, membrana elastica externa, the outer limit of the notochordal sheath; H, hæmal arch; N, neural arch; T, one of the concentric lamellæ characteristic of tectospondylous vertebræ. The cartilage next internal to the ring D is inner-zone cartilage, that between the rings D and E is outer-zone cartilage. Copied from HASSE (**15**, A, pp. 41, 44, 48).

The groups of fishes as delimited by HASSE are, with certain exceptions, natural groups, which is to be accounted for by the fact that his ultimate classification was not based upon the characters of the vertebræ solely; he wisely took also into consideration the characters of the placoid scales and teeth, the basal cartilages of the pectoral fin, and the presence or absence of an anal fin, and in difficult cases, where the features of the centra did not provide the confirmatory evidence that he needed, he was inclined to disregard, or at all events to belittle, the value of such evidence, with the result that his definitions regarding the patterns of the vertebral centra fail to be strictly applicable throughout the groups in question.

Cyclospondyly.

HASSE'S Plagiostomi cyclospondyli comprise the sharks and dog-fishes that are included within the first division of the family Squalidæ as delimited by REGAN (**29**, p. 723), namely, *Centroscyllium*, *Echinorhinus*, *Oxynotus* [*Centrina*], *Etmopterus* [*Spinax*], *Squalus* [*Acanthias*], *Scymnodon*, *Centroscymnus*, *Centrophorus*, *Scymnorhinus* [*Scymnus*], *Somniosus* [*Læmargus*], *Isistius*, and *Euprotomicrus*. According to HASSE'S briefer definitions (*e.g.*, **15**, A, pp. 42–44), and his diagrammatic figure (fig. 3, 1, above of the present paper), the calcification, excluding any superficial layers that may be present, consist of the double cone only, but in his more detailed accounts

(*e.g.*, **15**, B, p. 86) he notes the presence, in most of the Cyclospondyli that he examined, of a calcareous deposit in the innermost part of the outer-zone cartilage, closely applied, as a Belegschicht, to the double-cone calcification (see fig. 22, p. 372 of the present paper).

Tectospondyly.

In his introductory remarks upon the Plagiostomi tectospondyli* HASSE states that the uniform characteristic feature of all is a strengthening of the vertebral body by the deposition in the outer-zone cartilage of calcified layers concentrically arranged about the central double cone.† These layers he regards as a further development of the simple deposit (Belegschicht) that occurs directly external to the double cone in most of the Plagiostomi cyclospondyli. The vertebra of *Pristiophorus*, with a single hollow calcified cylinder outside the double cone, is, according to his view, the most primitive found in modern Plagiostomi tectospondyli (**15**, A, p, 46, and **15**, C, p. 97), and his text-fig. 5 (**15**, A, p. 44, reproduced here as fig. 3, 2, p. 338) is based upon it. He regards the genus *Pristiophorus* as a connecting link between the Cyclospondyli, on the one hand, and the bulk of the Tectospondyli on the other.

Squatina conforms well with his definition, there being several concentric calcified layers in the outer zone, separated from one another by uncalcified cartilage (fig. 25, p. 377 ; and HASSE, **15**, A, p. 47, and **15**, C, p. 129).

The first-formed calcification in the outer-zone cartilage is only exceptionally (*Pristiophorus*, *Squatina*) separate from the double cone ;‡ in the true Batoid fishes

* The Plagiostomi tectospondyli of HASSE comprise the Batoid fishes (Hypotremata of REGAN, **29**), *plus* the Pristiophorinæ and Squatinidæ ; the Plagiostomi cyclospondyli include the Squalidæ of REGAN *minus* the Pristiophorinæ ; the Plagiostomi asterospondyli include the Galeoidei of REGAN, together with the first three families of his Squaloidei, viz., the Cochliodontidæ, Hybodontidæ and Cestraciontidæ. In the first part of his monograph, HASSE (**15**, A) proposed the group-names Elasmobranchii polyspondyli and Plagiostomi diplospondyli for the Chimeroid fishes and the Notidanid sharks respectively, but he discarded the names in his later work (**15**, B) and called the groups Die Holocephalen and Die Notidaniden. The names, however, reappear in his Stammtafel II of 1885 (**15**, E).

† "Das gemeinsame Merkmal Aller besteht darin, dass sich concentrisch um den centralen Doppelkegel, also auch ringförmig um die Chorda Verkalkungsschichten ablagern, welche, der Aussenzone angehörig, den Wirbelkörper solide machen" (**15**, C, p. 97). In his preliminary account of the development of the vertebral centrum, before he has yet introduced the terms tectospondyli and asterospondyli, he writes :— "Entweder lagern sich um letzteren" (*i.e.*, the double cone) "concentrische Lagen verkalkten Knorpels (siehe Holzschnitt V), die sich in grösserer oder geringerer Ausdehnung bis an die Peripherie erstrecken können . . . . oder es gehen von dem centralen Doppelkegel Kalkstrahlen aus (siehe Holzschnitt VI)" (**15**, A, p. 25). Comparing the Tectospondyli and Asterospondyli with the Cyclospondyli, he writes :— "Die Plagiostomi tecto- und asterospondyli eine Weiterentwicklung insofern zeigen, als sich bei den ersteren um oder unmittelbar an dem centralen Doppelkegel, in der Mittelzone des Wirkelkörpers Kalkablagerungen in concentrischen Lagen geltend machen, während bei letzteren von dem centralen Doppelkegel in radiärer Richtung gesonderte Kalkstrahlen ausgehen" (**15**, A, p. 40).

‡ In the middle of its length, that is to say ; for the layer is confluent with the double cone at its anterior and posterior edges.

(= Hypotremata of REGAN) it is a Belegschicht, a calcified layer closely applied to the outer surface of the double cone (**15**, A, p. 46),* similar to that found in the Plagiostomi cyclospondyli. The later calcification in the Plagiostomi tectospondyli may be in the form of layers so uniform and dense as to produce a solid, compact mass nearly reaching to the surface of the centrum (*Pristis*, *Rhynchobatus*, etc., HASSE, **15**, C, Plate 16, figs. 56 and 52; Plate 19, fig. 3; Plate 20, fig 3; see also fig. 31, p. 385, of the present paper), or it may result in the production of radiating laminæ that present a star-like pattern in a transverse section of the centrum (*Raia*, fig. 4, B).

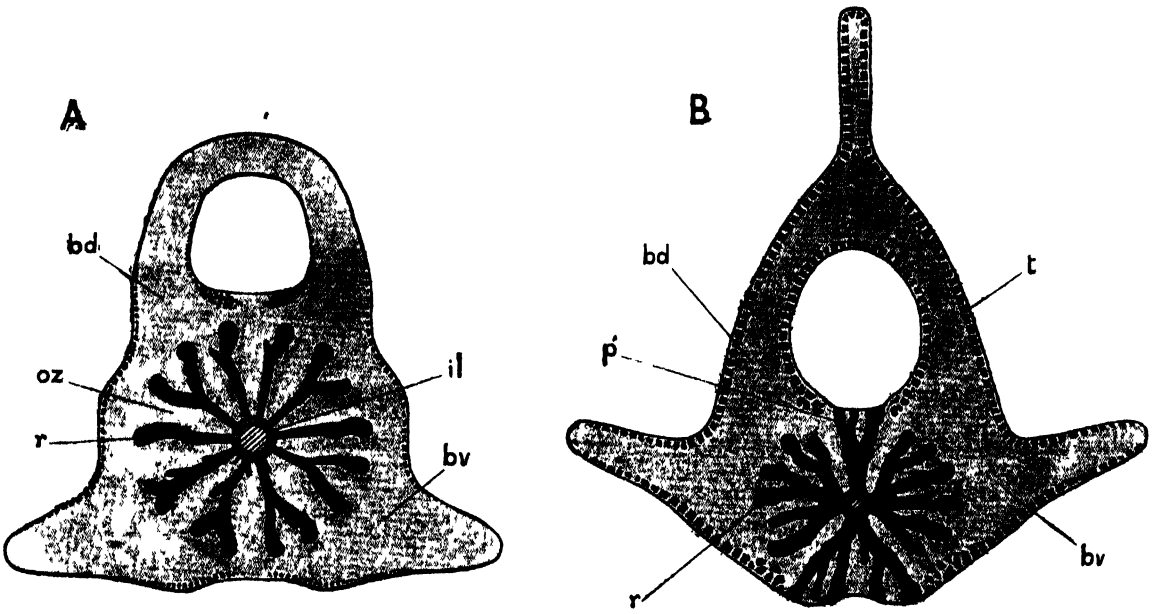


FIG. 4.—A, *Cestracion philippi*, × 2·4; B, *Raia marginata*, × 1·8. Transverse sections of trunk vertebræ cut midway between the anterior and posterior ends of the centrum. In the terms of HASSE (**15**) *Cestracion* exhibits asterospondyly, and *Raia* tectospondyly, but it will be noticed that the "star" is as well marked in the latter as in the former. (For explanation of the lettering, see p. 404.)

How HASSE came to include in his Plagiostomi tectospondyli, which, he states, are characterised by concentric calcified layers in their centra, Batoid fishes with strongly marked radiating laminæ, such as *Narcine brasiliensis* and *Trygonorhina fasciata* (**15**, C, Plate 23, fig. 21; Plate 15, fig. 49), as also *Raia marginata*, *Rhinobatus granulatus*, and *Torpedo narce* (fig. 4, B, above; fig. 30, A, p. 383; fig. 26, p. 379, of the present paper), is at first sight not clear, but it would seem that he derives such star-like forms of centra from the compact, *Pristis*-like condition by a failure in the calcification of certain radially disposed tracts of the outer-zone cartilage, for he explains the eight-rayed star or rosette of *Rhinobatus horkeli* (**15**, C, p. 114; Plate 15, fig. 27) as originating, by a process of incomplete calcification, from a compactly

* It might at first seem from the wording of the passage at the top of p. 46 that he regarded the Belegschicht as belonging to the middle zone, but a study of the context shows that he really considered it as the inner part of the outer zone.

calcified centrum such as that of *Rhinobatus thouini* (**15**, C, p. 110 ; Plate 14, fig. 14) ; and *Rhinobatus cemikulus* and *Trygonorhina fasciata* (**15**, C, Plate 15, figs. 41 and 49), are but further derivatives along the same lines of modification. On the other hand, it is to be observed that he derives the complex pattern seen in *Narcine brasiliensis* and *Torpedo marmorata* as due to the branching of the four rays, vertical and horizontal, of the simple cross found in *Astrape dipterygia* (**15**, A, p. 48 ; **15**, C, pp. 178, 175, 173 ; Plate 23, figs. 21, 11, 3).

It would seem, further, that HASSE changed his views during the interval that supervened between the publication of his Allgemeiner Theil in 1879 and the appearance of the second Lieferung of his Besonderer Theil in 1882 ; in the former he remarks (**15**, A, p. 47) that in the Trygon family the four oblique rays of the eight-rayed star seen in a transverse section of the centrum are calcifications of the arch-bases, and the vertical and horizontal rays are developed from external perichondrium ; but, in his contribution of 1882, he affixes the mark *a'* to one of the oblique rays of *Urolophus aurantiacus* and to one of the lateral rays of *Tæniura lymna* (**15**, C, Plate 19, figs. 9 and 16), the mark *a'* standing for "Aussenlage der Aussenzone." It is only fair to add that, on p. 146, he expresses himself as somewhat in doubt about the matter, owing to there being no traces of the membrana elastica externa recognisable, and to the fact that he was unable to investigate the matter developmentally ; he notes that the vertical and lateral rays reach the perichondrium, and are reinforced by it, and he remarks that KÖLLIKER was of opinion that they were "periostale Keile," *i.e.*, situated external to the sheath-cartilage.

As regards the concentric character of the deposit in those Plagiostomi tectospondyli that show stellate patterns, it is true that, in his figures of *Trygonorhina fasciata*, *Narcine brasiliensis*, and *Torpedo* sp. (**15**, C, Plate 15, fig. 49 ; Plate 23, figs. 21 and 14), he shows lines of the successive deposits, such that, if these are continued from ray to ray across the intervening tracts of cartilage, they yield a series of concentric circles, but, on the other hand, similar zones of growth are shown in his figures of the radiating laminæ that occur in a number of forms that he includes in the Plagiostomi asterospondyli, *e.g.*, *Cestracion philippi*, *Crossorhinus barbatus*, and *Ginglymostoma rüppeli* (**15**, D, Plate 24, fig. 8 ; Plate 25, fig. 6 ; Plate 26, fig. 21).

Asterospondyly.

HASSE states that the vertebræ of his Plagiostomi asterospondyli* are distinguished by the following characters. The innermost deposit of calcareous matter in the outer zone of the cartilage of the notochordal sheath is closely applied to the surface of the double cone ; from it there project four oblique radiating calcified laminæ directed towards the arch-bases, and there may be other radiating laminæ,

* The scope of HASSE'S Plagiostomi asterospondyli is given in the first footnote on p. 339.

commonly four in number. These last are horizontal and vertical in position, and are developed from the external perichondrium of the vertebral body.*

The description does not apply with any precision to the vertebræ of *Cestracion*, the first member that he includes in the group, for, in that genus, while the calcified rays are commonly (but not invariably) eight in number, they are not arranged according to the terms of his definition; instead of four of the rays being directed towards the middle of the arch-bases and the other four being disposed in horizontal and vertical positions, as, for instance, in *Mustelus* (fig. 8, A, p. 354), the eight rays are directed towards the *edges* of the four arch-bases (fig. 21, A, p. 370), and all of the rays are situated in the notochordal sheath, none being developed from the external perichondrium.

A similar relation might be supposed to obtain in early stages of development of *Ginglymostoma*, judging from his figure (**15**, D, Plate 26, fig. 19), in which the rays would seem to differ from those of *Cestracion* (**15**, D, Plate 24, fig. 7) solely in that the two ventrally directed rays are not separated from one another; but the figure is fallacious in that the membrana elastica externa is represented as pursuing a more or less circular course, as at *e* in fig. 18, A, p. 366, whereas careful study of embryonic material shows that it follows a sinuous course, as at *e e*. The inner parts of the lamellæ are at an early period continuous with the investing layer, and belong to the outer-zone region, but in the adult the bulk of the substance of the calcified lamellæ is of perichondrial origin, and the pattern of the transverse section comes to resemble that of *Stegostoma* (*cf.* **15**, D, *Ginglymostoma rüppeli*, Plate 26, fig. 21, and *Stegostoma fasciatum*, Plate 25, fig. 3). In *Chiloscyllium plagiosum* the eight rays begin to develop at the edges of the four arch-bases (fig. 17, E, p. 365), but they are *external* to the membrana elastica externa, and thus conform even less with his description of asterospondyly than do the rays of *Cestracion* and young *Ginglymostoma*.

In such forms as *Mustelus*, *Carcharias*, and *Sphyrna* the four diagonal rays, directed towards the middle parts of the arch-bases, are developed in the outer zone of the sheath-cartilage, and are thus comparable with the rays of *Cestracion*.

It would appear from a paragraph that occurs early in his monograph (**15**, A, p. 29, last paragraph) that HASSE originally intended his term asterospondyly to have reference mainly to the calcified rays that are developed within the outer-zone cartilage, *i.e.*, internal to the membrana elastica externa. It is only at the end of the paragraph that he alludes to the externally developed perichondrial calcifications, so that an inconsistency is introduced when he includes in his Plagiostomi asterospondyli such forms as the Lamidæ, for these, while they have the perichondrially produced wedges well developed, with numerous thin calcified lamellæ (fig. 9, *p'*,

* "Die Wirbelkörper haben . . . sich erstrecken" (**15**, A, p. 49); "Die ringförmige Verkalkung . . . Schwanzwirbelsäule darbietet" (**15**, D, pp. 183–184). (See also **15**, A, p. 29, last paragraph.)

p. 356), have the four oblique rays, directed towards the middle parts of the arch-cartilages, developed very feebly or not at all.

As showing yet further how unsatisfactory are HASSE's generalisations, it may be pointed out that in his remarks upon the Group Scyllia (**15**, A, p. 53) he says that the typical vertebræ of the group, exemplified by those of *Scyllium maculatum* and *marmoratum* (fig. 15, p. 363 of the present paper), exhibit eight rays developed in the outer-zone cartilage, namely, four diagonal rays directed towards the arch-bases, and four others, vertical and horizontal, which occur sometimes as single wedges, sometimes (as in *Scyliorhinus maculatus*) in the form of pairs of divaricated rays. His description of the origin of the eight rays, however, applies with equal precision to the vertebræ of *Dasybatis thalassia* and *Rhinobatus granulatus* (fig. 35, A, p. 391, and fig. 30, A, p. 383), in which there are four diagonal rays, connected at their inner ends with the double cone, directed towards the middle parts of the arch-cartilages, and not reaching the surface externally, and four other rays, vertical and horizontal, which are augmented at their outer extremities by perichondrial contributions. Yet the "Scyllia" are among HASSE's Asterospondyli, whereas the genera *Dasybatis* and *Rhinobatus* are Tectospondyli.

The basis of HASSE's third diagram, representing asterospondyly, is uncertain. He refers to the figure when describing the vertebræ of *Heptanchus* (**15**, A, p. 36), and it is to be noted that the figure shows six radiating lamellæ (fig. 3, 3, p. 338), which is the number commonly present in that genus. But *Heptanchus* is not one of HASSE's Plagiostomi asterospondyli ; it belongs to his group Plagiostomi diplospondyli. The only other Elasmobranch whose vertebra the figure at all resembles is *Cestracion*, but *Cestracion*, he says, has eight rays (**15**, A, p. 51). Elsewhere (**15**, D, p. 184) he remarks, nevertheless, that *Cestracion* is the most ancient of the living Plagiostomi asterospondyli, and presents evidence of affinity with *Heptanchus* in the characters of the calcified rays of the centrum, although the rays are more numerous.

The majority of the fishes that HASSE includes among the Plagiostomi asterospondyli have calcified lamellæ or wedges that are developed to a large extent, if not entirely, by the superficial perichondrium of the centrum ; these structures are not introduced into the figure, and it fails to be representative in consequence of the omission. The whole of the calcified structures that he shows in the figure are situated within the limits of the membrana elastica externa, *i.e.*, they are in the substance of the chondrified notochordal sheath.

HASSE's second diagram (fig. 3, 2, p. 338), representing tectospondyly, is based upon the vertebræ of *Pristiophorus* (*vide supra*). It shows a single calcified tube, external to the double cone and united therewith at its anterior and posterior ends only. That HASSE should have chosen the vertebra of *Pristiophorus* as his most typical example of tectospondyly, to be contrasted with vertebræ having radiating lamellæ; proves to be most unfortunate in view of REGAN's discovery of radiating lamellæ in the Pristiophorid, *Pliotrema warreni*. See fig. 24, B, p. 375.

Views of Later Authors.

SMITH WOODWARD, referring to HASSE'S employment of the characters of the vertebral centra of the Tectospondyli and Asterospondyli as a basis of classification, states that the features are to such an extent distinctive that he ventures to adopt the taxonomic arrangement (**32**, p. xxvi). He agrees with HASSE in regarding the Squatinidæ and Pristiophoridæ as surviving members of the ancestral stock that produced the Rays. But the absence of the anal fin in the Spinacidæ and the low position of the gill-clefts in some forms, such as *Acanthias*, lead him to regard the family as more related to the ancestral stock of the Rays than to that of the Sharks that possess vertebræ showing a star-like pattern in transverse section (*ibid.*, p. xxv).

SMITH WOODWARD does not admit cyclospondyly as a valid basis for a taxonomic group of fishes; there are, he says, known cyclospondylic members of each of the groups Tectospondyli and Asterospondyli (*ibid.*, p. xxvii). While in the opinion of SMITH WOODWARD the Squalinæ are derived from the base of the Tectospondylic series, *Palæospinax* is a Cestraciont, an early representative of the Asterospondyli; it has an anal fin, yet the vertebræ exhibit a simple double cone without radiating laminæ (*ibid.*, p. xxvii). "The *degree* of development of the vertebral centra," he states, "is of small importance." *Hybodus*, for instance, is an undoubted Cestraciont, yet there are no calcified structures in the centra of the vertebræ. "The acceleration of vertebral development," he adds, "and the retardation of the same, are singular features apparently having little correspondence with the specialization or otherwise of characters still more likely to change."

SMITH WOODWARD'S classification is one in which HASSE'S terms Tectospondyli and Asterospondyli are employed to designate sub-orders of the order Selachii, but with altered significance; the Tectospondyli embrace not only the Rays and the families Squatinidæ and Pristiophoridæ, but the family Spinacidæ (= Squalinæ) as well; and the Asterospondyli are enlarged in scope by the inclusion of the Notidanidæ (*ibid.*, pp. xxxiv–xlv).

GILL employs the group-names Cyclospondyli, Tectospondyli and Asterospondyli, but not strictly in HASSE'S sense; his Cyclospondyli are HASSE'S Diplospondyli, *i.e.*, the Notidanidæ or Hexanchidæ ('Bull. U.S. Nat. Mus.,' No. 16, 1883, p. 967); his Tectospondyli include the sharks without anal fin (HASSE'S Cyclospondyli), and his Asterospondyli exclude the Cestraciontidæ (which are separated off as the Prosarthri), and include the Pristiophoridæ and Squatinidæ, which were part of HASSE'S Tectospondyli. The rest of HASSE'S Tectospondyli—the Batoid fishes—GILL terms the Hypotremi ('Mem. Nat. Acad. Sci.,' vi, mem. 6, Washington, 1893, pp. 129–130).

GREGORY adopts a compromise; in his sub-orders Cyclospondyli and Tectospondyli he includes the same fishes as HASSE did, but his order Asterospondyli differs from HASSE'S Asterospondyli in that it excludes the Cestraciontidæ ('Ann. N.Y. Acad. Sci.,' vol. xvii, No. 3, part 2, September, 1907, p. 446).

Regan in 1906 (**29**, pp. 733–4), after referring to the predominance of the concentric laminæ in the Tectospondyli and of the radiating laminæ in the Asterospondyli, observes that "these terms have no practical application; both groups include types in which the secondary calcification of the vertebral centra has no laminar structure (*e.g.*, *Rhynchobatus*, *Oxyrhina*),* and others in which it is deposited as a series of concentric laminæ (*Squatina*, *Cetorhinus*); also in both are forms in which the calcification presents a radiating pattern in cross section (*e.g.*, *Narcine*, *Orectolobus*)." He proposes new definitions for Hasse's terms. "Asterospondylic centra," he writes (**29**, p. 737), "may be defined as those in which the secondary calcification leaves four principal uncalcified areas radiating from the central double cone to the bases of the neural and hæmal arches, and are characteristic of the sub-order Galeoidei, although in the Scyliorhinidæ a series of modifications set in which culminate in a complete reversion to the cyclospondylic type in the genera *Pristiurus* and *Pseudotriacis*. Tectospondylic centra are those with well-developed secondary calcifications not arranged on the asterospondylic plan. Hasse has applied this term to the various types of centra found in the Batoidei, and in *Squatina* and *Pristiophorus*, and it is impossible to give any definition which will include these and exclude *Cestracion*. Probably also the so-called asterospondylic centra of some Hybodonts would have to be included."

The new delimitation thus proposed is unfortunate in that the first of the new definitions renders Hasse's diagrammatic figure of asterospondyly (fig. 3, 3, p. 338), no longer applicable to "asterospondylic" vertebræ; and the definition is so worded that some of the caudal vertebræ of Batoids (*e.g.*, *Torpedo*, fig. 26, B, p. 379), would have to be regarded as asterospondylic. The difficulties in the way of consistent new definitions of Hasse's terms are almost insurmountable, and it would tax one's ingenuity to the utmost to draft short descriptions that would differentiate the vertebræ of *Mustelus* and *Dasybatis* (fig. 8, A, p. 354, and fig. 35, A, p. 391), as asterospondylic and tectospondylic respectively. A certain amount of confusion is bound to result from the retention of the terms with new meanings, and it is far preferable to discard them altogether (see p. 346).

Schauinsland, in his admirable summary of the views of Kölliker, Goette, and Hasse, states that the secondary calcifications in the vertebral centra have "eine weitgehende systematische Würdigung" (**30**, p. 405).

Goodrich† writes: "That these characters of the centrum are of considerable taxonomic value there can be no doubt, but the distinction between the various types does not seem to be as clear and sharp as was supposed."

Graham Kerr in 1911 writes‡: "The arrangement of the calcified tracts shows differences which are of taxonomic importance."

* *Vide infra*, p. 358.

† 'Treatise on Zoology,' E. Ray Lankester, Part IX, fasc. 1, London, 1909, p. 135.

‡ 'Encyclopædia Britannica,' Ed. XI, vol. 24, Article "Selachians," p. 594.

Terms Asterospondylous and Tectospondylous better Discarded.

For the reasons given in the previous section, it is deemed advisable to discontinue the use of the terms asterospondylous and tectospondylous; the term cyclospondylous is less open to objection than these, but there is no great advantage to be gained in retaining it if the other terms of the series are no longer employed. The attempt to introduce a new series of descriptive terms, more accurate and more consistent in their application than those which it is proposed to discard, has not met with any great measure of success, for while some vertebræ offer characters that can be satisfactorily expressed by single descriptive words, there are others that do not lend themselves readily to the process; they fail to fit into a general scheme, because they do not present characters that can be regarded as specially distinctive.

As belonging to the first kind, those with features that can be pointed to as distinctive are *Cestracion*, *Squatina*, and *Squalus*. The centra of *Cestracion* might, for instance, be described as *actinospondylous*—they have calcified lamellæ that radiate outward from the primary double cone; the rays occur solely in the outer-zone cartilage, they are approximately equidistant, and their disposition is not influenced in any way by the arch-cartilages (fig. 4, A, p. 340). Those caudal vertebræ of *Heptanchus* that show a star-like pattern fall into the same category (fig. 5, A, p. 348).

The centra of *Squatina* exhibit a series of thin calcified lamellæ, which are set in a concentric manner around the primary double cone (fig. 25, p. 377). They occur in the outer-zone cartilage, and they are not affected by the arch-cartilages, in these respects differing from those of a young *Cetorhinus* (fig. 13, A, p. 360). The concentric lamellæ increase in number as the vertebra grows, the outermost lamella being the latest; they thus come to resemble the "rings" of annual growth in the section of the wood of a tree-trunk, and the vertebræ might accordingly be termed *xylospondylous*. An eminent Greek scholar was good enough to suggest *prasospondylous*, to suggest the coats of a leek, but the resemblance fails in that in the leek and onion the outer coats are the oldest, not the most recently formed.

In *Squalus*, *Spinax*, and *Oxynotus* the double cone is covered on its external surface with an investing layer of secondary calcification, developed from the inner part of the outer zone cartilage (fig. 22, p. 372). This calcified body, when exposed by scraping away the uncalcified cartilage from it, is seen to have a smooth surface, free from radial lamellæ; the term *litospondylous* applied to such a vertebra would be more appropriate than cyclospondylous, for the latter, largely from error—for Hasse distinctly refers to a Belegschicht—has come in course of time to signify that the central calcified structure consists of the double cone solely. Or, alternatively, the term *haplospondylous* might be used, signifying that the calcified parts are of single construction.

The absence of the double cone and of secondary calcifications from the vertebral

column of *Hexanchus*, *Echinorhinus* (fig. 23, p. 373), and *Somniosus microcephalus* may, for the reasons given elsewhere, be regarded as a condition of degeneration, and not a primitive one. Having lost their calcifications, the vertebræ might be termed *lipospondylous*.

If a descriptive term were desired for the compact secondary calcification exhibited by the centra of *Rhynchobatus* and *Pristis* (fig. 31, p. 385), *sympektospondylous* might be suggested; but there is nothing to be gained by the introduction of the term, for the condition is so nearly approached by the vertebræ of some species of *Rhinobatus*, other species of which genus exhibit a characteristic eight-rayed star. Compare, for instance, the series *Rhinobatus thouini*, *horkeli*, and *cemiculus*, given by HASSE (**15**, C, Plate 14, fig. 14; Plate 15, figs. 27 and 41).

In the Carcharidæ there are diagonal calcified lamellæ (*Sphyrna*, fig. 8, B, p. 354) for which the prefix *chiasto-* might be appropriate. And in some genera, such as *Galeus* and *Mustelus*, the calcified intermedialia present the form of a massive cross with vertical and horizontal arms (fig. 8, A), suggesting the use of the prefix *stauro-*. But it would be difficult so to define a single word describing the structure of the vertebræ of a Carchariid shark that it would exclude the vertebræ of Batoids like *Trygonorhina* (HASSE, **15**, C, Plate 15, fig. 49).

The Batoids, indeed, would seem to defy all attempts to get them to fall naturally into any scheme such as that which HASSE set himself to propound. The differences observable in some cases between the vertebræ of the caudal and trunk regions are not merely differences in pattern, but differences in origin, as witness the horizontal rays, which in the trunk region do not reach the surface, and are not reinforced by perichondrially produced increments as are the horizontal rays in the caudal region (*Rhinobatus*, fig. 29, A, p. 382, and fig. 30, A).

The suggestions, therefore, that are set forth in the preceding paragraphs are made, not with a view to the adoption of the terms, but rather to show that a comprehensive and consistent terminology is not possible; it is here definitely recommended that the employment of the terms cyclo-, astero-, and tectospondylous should be discontinued, but the terms mentioned in the paragraphs above are equally to be rejected.

DETAILED OBSERVATIONS.

The letterpress of the various sections of the paper included under this heading has been made as brief as possible. The numerous text-figures drawn in illustration of the facts observed are treated rather diagrammatically, and they convey to the reader the author's views far more readily than a copious descriptive text. They are therefore left to a large extent to speak for themselves.

Chlamydoselachidæ.

No specimens of *Chlamydoselachus* were examined in the course of the investigation. This was not because material could not be procured, but because the account

given by GOODEY* in 1910, taken in conjunction with the earlier description by GARMAN,† goes to show that the vertebræ are of little interest in the present connection. According to GARMAN (pp. 12–13), the notochord is unconstricted for the greater part of its length, although in the anterior region there are vertebral constrictions, with calcifications resembling those of *Centrophorus*. Behind this region the limits of the vertebræ can only be made out from the neural and other plates. GOODEY (pp. 553–561) finds double-cone calcifications in the caudal vertebræ as well as in those of the branchial region. It is probable that the examination of a large number of specimens would show that there is a good deal of individual variation within the species, and that the condition of the vertebral column is degenerate, not primitive.

Hexanchidæ.

The material available for study consisted of the vertebral column of a half-grown specimen of *Heptanchus cinereus*, about a metre in length, and the branchial region of the vertebral column of another specimen, of about the same size.

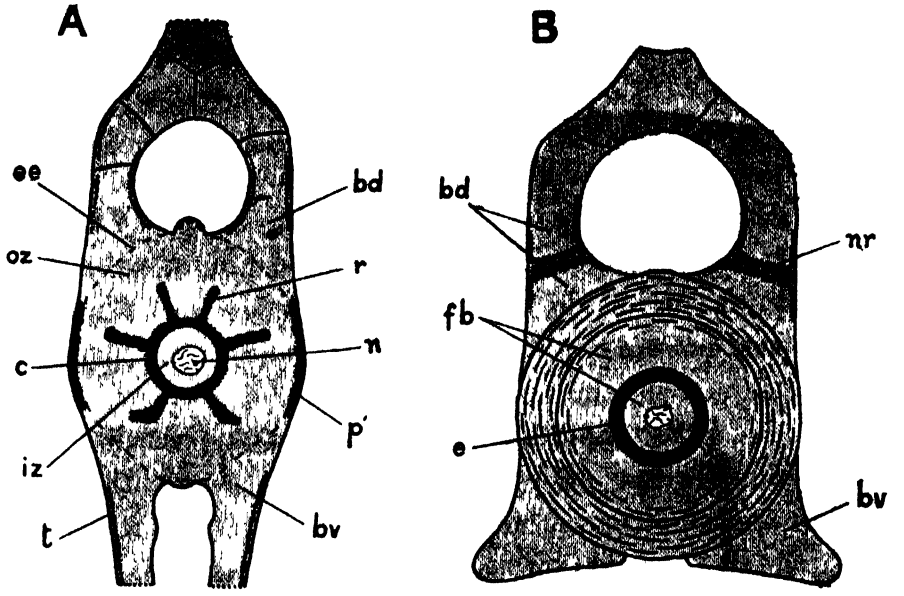


FIG. 5.—*Heptanchus cinereus*, half-grown fish, 1 metre long. A, vertebra from region of caudal fin, at a point two-thirds of the length of the caudal fin from its posterior end, × 5. B, mid-trunk vertebra, × 3·5.

e, calcified ring representing the last remnant of the double cone, *cf. e* in fig. 6; *fb*, fibro-cartilaginous septum, a modification of the outer-zone and inner-zone cartilage. (For explanation of other lettering, see p. 404.)

In *Hexanchus* there are no double cones or other calcified parts of the centra (KÖLLIKER, **20**, p. 51; HASSE, **15**, B, p. 43), and the positions of the "centra" can only be recognised by the presence of large transverse septa of fibro-cartilage occurring at intervals within the tubular sheath that surrounds the notochord. The

* GOODEY, T., 'Proc. Zool. Soc.,' 1910, pp. 540–571.

† GARMAN, S., 'Bull. Mus. Comp. Zool.,' Harvard Coll., xii, 1, Camb., Mass., 1885–6.

same applies to *Heptanchus*, except that, in the caudal region, some of the vertebræ have double cones and radiating calcified lamellæ. The conditions found in these two genera point to the vertebral column being in a degenerate, and not in a primitive, condition, and indicate that the vertebræ of *Hexanchus* are more degenerate than those of *Heptanchus*. It is to be noted that in *Notidanus eximius*, of the Jurassic, the centra are more calcified than in the modern *Heptanchus* (HASSE, **15**, B, p. 52).

Accepting the general conclusion that the caudal vertebræ of a shark exhibit a less specialised condition than those of the trunk region (p. 317), the presence of double cones and radiating lamellæ in some of the hinder caudal vertebræ of *Heptanchus* implies that the absence of such calcifications from the trunk region is due to degeneration.

The structure of the double cone and radiating lamellæ of the caudal vertebræ of *Heptanchus* has been described by KÖLLIKER (**19**, Plate 2, figs. 2 and 3) and HASSE (**15**, A, p. 36, and **15**, B, p. 49, and Plates 6 and 7), and both of these authors have noted that, as one passes forward in the caudal region, the double cones shorten, and become merely rings of calcified cartilage. The specimen studied in the course of the present investigation seems to be remarkable, in that the rings, usually confined to the caudal region, extend through the whole of the trunk as well. Vertebræ from the branchial region of another fish of about the same size failed to show them.

The transition from the double cone to the ring is explained in fig. 6 (p. 350). The double cones of *Heptanchus* are present in their most complete form in that part of the caudal fin which is two-thirds of the length of the fin from its posterior end (fig. 6, 1, and fig. 5, A). The outer-zone cartilage (fig. 6, 1, *a*) is hyaline in character, and contains six radiating calcified lamellæ,* connected at their inner edges with the double cone, and not reaching the surface of the centrum (fig. 5, A, *r*). The remnants of the membrana elastica externa are readily recognisable (*e e*), and they go to show that the sheath-cartilage is of considerable bulk, and that the radiating lamellæ are calcifications of that cartilage.

At the root of the caudal fin the double cones are much shorter than in the region above specified, and the intervertebral ligaments are longer (fig. 6, 2). This antero-posterior compression of the double cone becomes more pronounced as one passes farther forward, until the calcified structure comes to consist of a two-layered circular plate (fig. 6, 3, *d*). Still farther forward, in the cloacal region, the more peripheral parts of the calcified structure disappear, and the inner part remains as a calcified ring, embedded in a transverse septum (fig. 5, B, *e*; fig. 6, 4, *e*). In the vertebræ of the tail there is an investing layer, a thin calcified deposit in outer-zone

* The rays are not necessarily regular; they may be of unequal lengths, and may vary in number; KÖLLIKER mentions eight, and HASSE figures vertebræ with six and four respectively (**15**, B, Plate 6, figs. 11, 12).

cartilage, immediately external to the double cone; it is shown in fig. 5, but not in fig. 6, which is more diagrammatic.

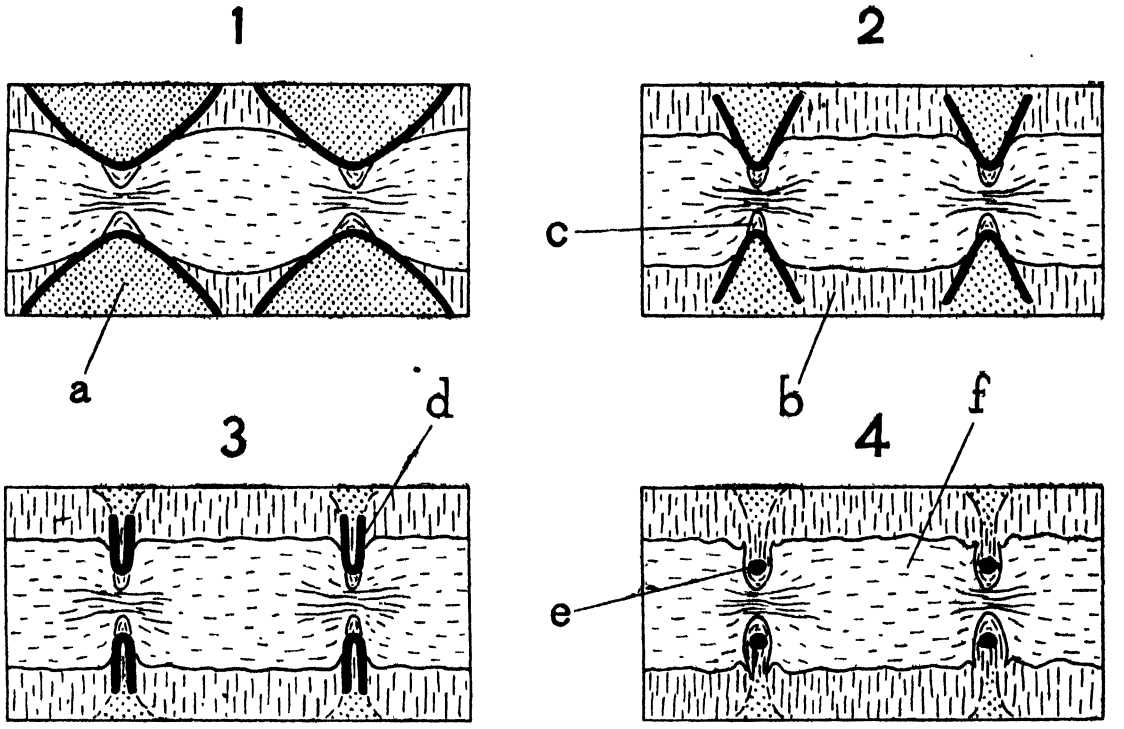


FIG. 6.—*Heptanchus cinereus*. Vertical sections through two consecutive centra, showing how the double cone, found in the vertebræ of the caudal fin, passes into the simple ring found in the more anterior vertebræ. To facilitate comparison the centra are represented as of the same size in the four regions selected. The upper edge of each figure marks the floor of the neural canal, the lower edge the roof of the hæmal canal.

1, vertebræ at the level of the hind end of the large anterior lobe of the caudal fin. 2, vertebræ at the root of the caudal fin. 3, vertebræ a short distance in front of the caudal fin. 4, vertebræ still farther forward, cloacal region.

a, hyaline cartilage (outer-zone cartilage); *b*, fibrous tissue, the greatly elongated intervertebral ligament; *c*, fibrous septum, a product of the inner-zone cartilage—it is traversed in the middle by the remains of the notochord; *d*, a two-layered circular plate, with fibro-cartilage between the two layers of the plate; it represents the double cone, antero-posteriorly compressed; *e*, calcified ring, the last remnant of the double cone (see also fig. 5, B, *e*); *f*, watery jelly, the remains of the notochord.

The arch-cartilages have calcified tesseræ, and the fact that these are better developed in the caudal than in the trunk region is a further argument in support of the view that the trunk vertebræ are in a degraded, and not in a primitive condition. At the sides of the caudal vertebræ, on a horizontal level with the middle of the centrum, are calcified tracts that lie just external to the membrana elastica externa (fig. 5, A, *p'*). These lateral tracts I regard as having been produced by the activity of the superficial perichondrium, and not by a fusion of neural and hæmal arch-cartilages; they are, in fact, intermedialia, but, instead of being bulky wedges as in the Carchariidæ, they are reduced to the form of thin plates. KÖLLIKER'S

longitudinal section (**19**, Plate 2, fig. 2) is taken horizontally, and shows the plates, but fig. 6, 1, of the present paper represents a vertical section, and so the plates are not seen.

Carchariidæ.

In REGAN'S classification (**29**) the families of the Galeoidei follow in the order Odontaspididæ, Lamnidæ, Orectolobidæ, Scyliorhinidæ, Carchariidæ, but for reasons that will presently be apparent, it is advisable here to commence with the Carchariidæ, and to leave the Orectolobidæ till the last.

The material available for study consisted of:—

Carcharias laticauda, two young specimens in alcohol, 380 mm. long, and one young (? embryo), 277 mm. long, in the collection at the Roy. Coll. Science.

Carcharias walbeehmi, 810 mm. long, from Tuticorin, S. India, vertebral column in alcohol, presented by J. HORNELL, Esq.

Carcharias (*Scoliodon*) sp., adult, a few dried vertebræ, Brit. Mus. (Nat. Hist.).

Carcharias, sp. (? embryo), 133 mm. long, no locality, Brit. Mus. (Nat. Hist.).

Galeus canis, three adults, purchased from Marine Biol. Lab., Plymouth; also young (? new-born), 230 mm. long, no locality, Brit. Mus. (Nat. Hist.).

Triacis semifasciatus, young, 355 mm. long, no locality, Brit. Mus. (Nat. Hist.).

Mustelus vulgaris, five adults, and one late embryo, 286 mm. long, purchased from the Marine Biol. Lab., Plymouth.

Sphyrna tudes, young, 480 mm. long, and *Sphyrna blochii*, two, young, 510 and 430 mm. long, S. India, vertebral columns in alcohol, presented by J. HORNELL, Esq.

Hemigaleus balfouri, 760 mm. long, S. India, vertebral column in alcohol, presented by J. HORNELL, Esq.

The early development of the vertebræ of *Mustelus* has been already described in detail by GOETTE, HASSE, KLAATSCH and GÖPPERT, but most of the embryos studied by them are too young to be of great interest in the present connection. KLAATSCH in 1893 (**18**, II) described the vertebral structures in embryos measuring 30, 40, 45 and 55 mm. long, and in the first two he demonstrated the immigration of cartilage-forming cells from the arch-bases into the notochordal sheath. The embryos described by HASSE in 1882 (**15**, D, p. 281) measured 18 and 40 mm. in length, and those described by him in 1892 (**16**) from 12 to 40 mm.; GÖPPERT'S embryos measured 23, 48 and 70 mm. (**11**, Plate 14). The embryos studied by GOETTE (**13**) were larger (100 and 220 mm.), and more nearly approached the ripe embryo examined in the present inquiry (286 mm.). Among other young Carchariids that have been studied may be mentioned *Galeocerdo tigrinus* (HASSE, **15**, D, p. 259 and Plate 36, fig. 15), *Loxodon macrorhinus* (197·5 mm., HASSE, **15**, E, p. 23 and fig. 3), *Carcharias lamia* (200 mm., GOETTE, **13**, Plate 31, fig. 41); and during the present inquiry there were examined *Carcharias laticauda* (277 mm.) and *Galeus canis* (230 mm.).

The figure of *Galeus* shown at C in fig. 7, p. 352, agrees in general structure with

that of *Mustelus* shown in HASSE's Plate 40, fig. 7 (15, D), although the diagonal rays are longer. The course of the membrana elastica externa is almost identical in the two cases; its outline is roughly octagonal, and it is superficial in four places, and marks off the arch-bases where it is not superficial. The young *Galeus* described by HASSE (15, D, p. 264 and Plate 38, fig. 3) is considerably older, and in its degree of development agrees nearly with the *Mustelus* here figured as fig. 7, A. The illustration given by HASSE is not clear; but taking the figure in conjunction with the text, it would seem that the author regarded the membrana elastica externa as stretching straight across the arch-bases, whereas there is incontestible evidence in *Mustelus* that, while the membrane keeps external to the diagonal calcified lamellæ (fig. 7, A, *d*), it occupies an internal position with regard to the perichondrial calcifications (*p'*). It follows the course rather of the line that HASSE marks *g* (Grenzzone), although not precisely.

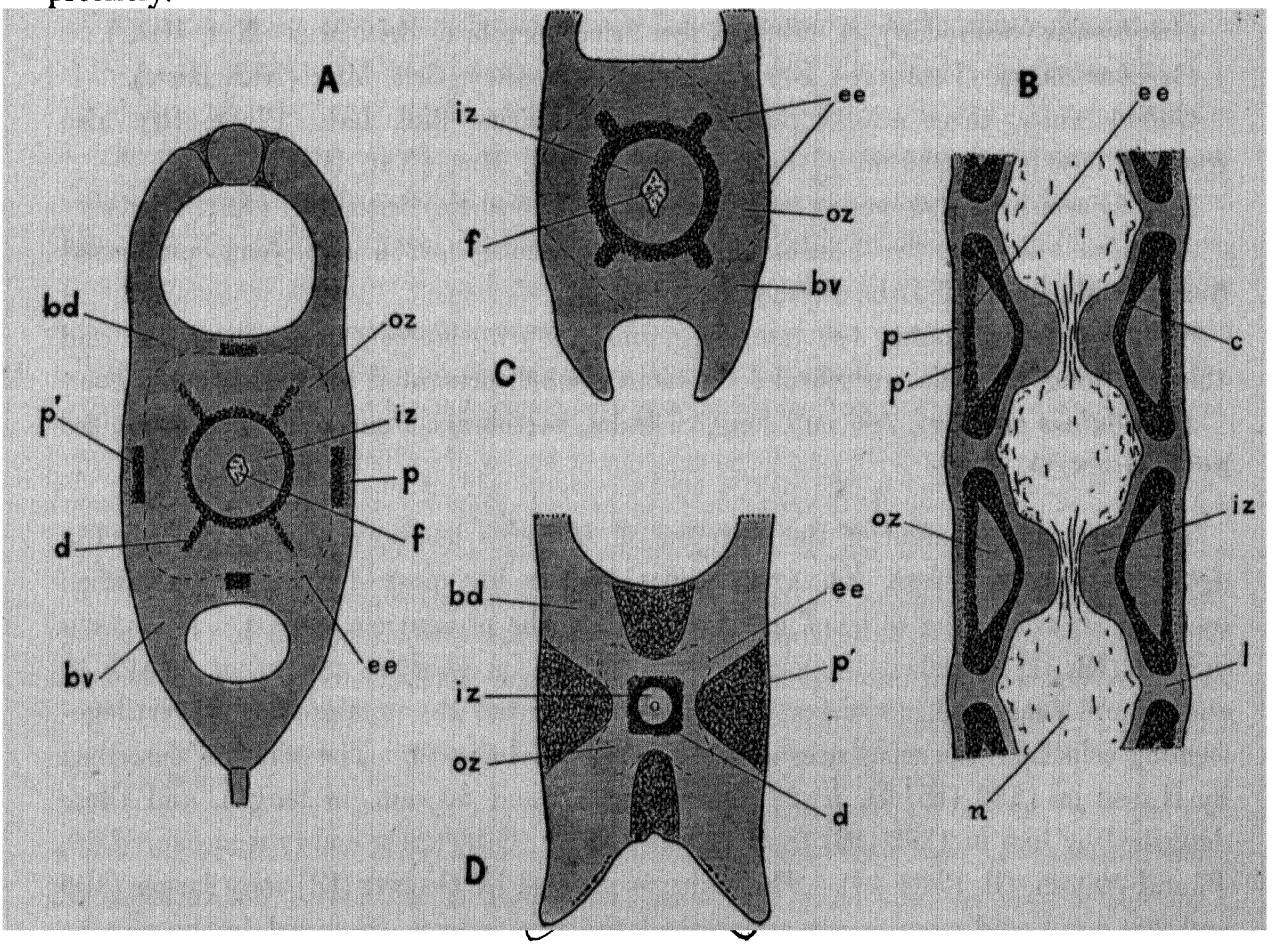


FIG. 7.—A, *Mustelus vulgaris*, late embryo, 286 mm. long, caudal vertebra from region of second dorsal fin, × 20. B, horizontal section of vertebræ from the same region, × 20. C, *Galeus canis*, young, 230 mm. long, vertebra from region between pelvic and second dorsal fins, × 18. D, *Carcharias latucauda*, young, 277 mm. long, mid-trunk vertebra, × 18. (For explanation of the lettering, see p. 404.)

It may here be remarked parenthetically that in the opinion of the present writer the Grenzzonen of HASSE have no morphological value; their limits are defined by

differences in the texture of the cartilage due in some cases to an unimportant transition from hyaline to fibro-cartilage, and in some cases to unequal shrinkage of the cartilage in the process of embedding and section cutting. The unequal shrinkage results from the rigidity of the calcified parts being greater than that of the uncalcified parts of the centrum, even after the process of decalcification; and it is significant that in some instances a Grenzzone that is distinguishable in sections prepared by the paraffin-embedding method, is not seen in hand-cut slices. The matrix of the cartilage in the regions where the strains and stresses are greatest is altered in some way that results in its staining differently from the parts of the cartilage that are less affected. In a few cases a Grenzzone is a "growing zone," a tract of small-celled, actively dividing cartilage, sometimes rendered more conspicuous by the inclusion within it of blood-vessels that penetrate from the superficial connective tissue.

The intermedialia of the late embryo of *Mustelus* (fig. 7, A) are very distinct from the arch-cartilages, their cells being smaller and more crowded; the inner part (p') is calcified, but not the more superficial part (p). The longitudinal section (B) shows the antero-posterior extent of the membrana elastica externa ($e\,e$), and illustrates how the inner-zone cartilage ($i\,z$) thins out abruptly towards the ends of the centrum. The double-cone calcification is seen to be thicker at the ends than at the middle, and the intermediale is confluent with the double cone at its anterior and posterior ends.

The section of the vertebra of *Carcharias laticauda*, shown in fig. 7, D, is interesting as showing the relatively late development of the diagonal calcified lamellæ, for in *Mustelus* (fig. A) the diagonals are well calcified at the time when the intermedialia begin to develop, and in *Galeus* (fig. C) they are present even before the perichondrial tracts of the intermedialia have begun to be active, the membrana elastica externa in those four regions being still superficial in position. Notwithstanding the late development of the diagonal calcified lamellæ in *Carcharias laticauda*, the diagonal out-pushings of the outer-zone cartilage are as well marked as if the lamellæ were already there.

HASSE'S figure of a young *Galeocerdo* (**15**, D, Plate 36, fig. 15) is peculiar in showing the diagonal lamellæ unconnected with the double cone; the absence of the upper intermediale, also, strikes one as strange.

As will be seen, by comparing the young and adult conditions of the vertebra of *Mustelus* (fig. 7, A, and fig. 8, A), the intermedialia broaden with age. In the adult they have the form of wedges (Periostale Keile of HASSE), each with the thin edge directed towards the axis of the centrum, and the base still in contact with the superficial connective tissue. In old specimens the growth of the base, superficially or tangentially, is greatly in excess of the radial growth of the wedge, and the "side" of the wedge, that face which abuts upon the basidorsal or basiventral cartilage, is curved instead of being flat. In old specimens of *Galeus*, this proceeds

to such an excessive degree that the base of the wedge comes to lie external to some of the tesseræ.

As regards the growth of the intermedialia in the radial direction, it is important to bear in mind that the growth is centrifugal, the latest increments being those that are most external and superficial. A casual glance at the sections might perhaps suggest that the intermedialia push their way inward, and in this way produce the curvature of the membrana elastica externa in the horizontal and vertical planes; and it is not quite clear that SCHAUINSLAND did not intend to convey such a meaning when he wrote* that they "dringen erst nachträglich in sie [the membrana] hinein." As an actual fact, the distance between the inner portions of the two lateral intermedialia is slightly greater in the adult than in the late embryo examined.

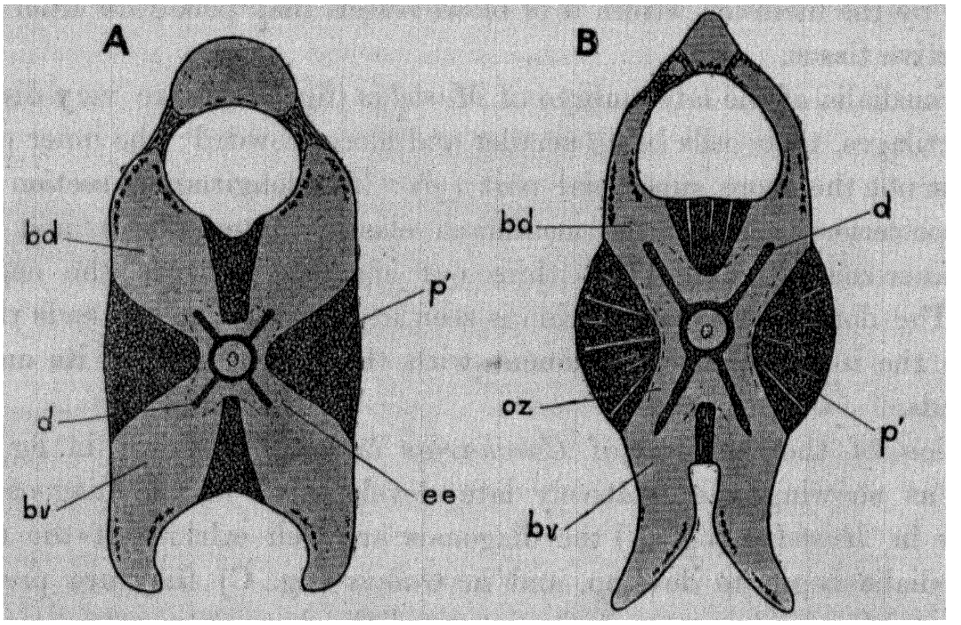


FIG. 8.—A, *Mustelus vulgaris*, adult, post-cloacal vertebra, × 5. B, *Sphyrna blochii*, young, 510 mm. long, pre-cloacal vertebra, × 7. (For explanation of the lettering, see p. 404.)

Another rather dubious remark of SCHAUINSLAND'S is that the diagonal calcified lamellæ "entstehen von der Mittelzone aus" (**30**, p. 405). This might mean that they belong morphologically to the double cone, and have their origin, like the double cone, in middle-zone cartilage, or it might mean that they are calcifications of the outer-zone cartilage which, beginning their development against the double cone, grow centrifugally away from it. The latter is the correct view; they are solely outer-zone structures.

In *Mustelus*, the remnants of the membrana elastica externa can be distinguished

* SCHAUINSLAND, **30**, p. 405; possibly after GOETTE, who says (**13**, p. 491) that the intermediale "druckt dabei die Aussenzone entsprechend ein, worauf seine Verkalkung sich in den Knorpel der letzteren fortsetzt, bis sie dem knöchernen Doppelkegel nahe kommt oder ihn theilweise erreicht. Auf diese Weise entstehen die schon erwähnten senkrechten und horizontalen Knochenleisten bei *Mustelus* und *Carcharias*."

in sections of vertebræ of the adult fish (fig. 8, A, *e e*), although the portions actually at the extremity of the diagonal lamellæ are missing. In one vertebra, however, the outward rate of calcification of the diagonal lamella exceeded, as it were, the rate of outward bulging of the sheath-cartilage, and the end-portion of the membrane became caught or entangled in the lamella, as is shown in the right upper quadrant of fig. 8, A. All four quadrants of the vertebra in question showed the phenomenon; the next vertebra to it exhibited the usual relations; in order to avoid the necessity of a separate figure, the exceptional relations are here illustrated in one of the quadrants of a vertebra which was in point of fact normal throughout. The exceptional condition is of interest in showing the disregard exhibited by a growing calcification for the mode of origin of the cartilage in which it is proceeding; it is immaterial that the calcification, begun in outer-zone cartilage, should be confined to that cartilage; it may proceed unchecked into the arch-cartilage external to the limiting membrane. Another instance of such disregard of the origin of the cartilage is given in the case of *Pliotrema* (p. 375).

In the adults of *Carcharias* and *Sphyrna* the lateral intermedialia are very obtuse, the base of the wedge being large and the radial extent relatively small. The calcification is usually compact in the former genus, but in *Sphyrna* there are radial rod-shaped tracts of uncalcified cartilage in the intermedialia (fig. 8, B), with blood-vessels penetrating into them. Similar tracts of uncalcified cartilage are shown in HASSE'S figure of a young *Prionodon melanopterus* (**15**, D, Plate 39, fig. 12).

It is in *Carcharias*, *Sphyrna*, and *Galeocerdo* that the diagonal calcified lamellæ attain their greatest radial extent; in some specimens of *Galeus* and *Mustelus* they are very feebly developed.

In the caudal region of *Galeus*, where diplospondyly prevails, the centra are alternately long and short; the patterns of the calcified parts of the long and short centra, as seen in transverse section, prove on examination to be the same.

Lamnidæ and Odontaspididæ.

The material of these families available for study consisted of:—

Lamna cornubica, adult, disarticulated skeleton in formalin, in the collection at the Royal Coll. Science; and dried vertebræ of a young specimen, about 900 mm. long, Brit. Mus. (Nat. Hist.).

Carcharodon rondeletii, a few vertebræ prepared by the glycerine jelly method, in the collection at the Royal Coll. Science, apparently from Prof. J. PARKER, Otago.

Cetorhinus maximus, dried vertebræ of adult, Shanklin, Isle of Wight, Brit. Mus. (Nat. Hist.); and transverse section of trunk vertebra of a young specimen, Brit. Mus. (Nat. Hist.); also another slice, possibly from the same fish, in the Museum of the Roy. Coll. Surgeons.

Alopias vulpes, two dried tails, no history, Brit. Mus. (Nat. Hist.).

Odontaspis americanus, young, about 900 mm. long, in alcohol, Rio Grande do Sol, Brit. Mus. (Nat. Hist.).

Scapanorhynchus (*Mitsukurina*) *owstoni*, adult, vertebræ in formalin, Japan, Brit. Mus. (Nat. Hist.).

Scapanorhynchus lewisi, two vertebræ from the Upper Cretaceous of Sahel Alma, Mount Lebanon, Geol. Dept., Brit. Mus. (Nat. Hist.).

In the families Lamnidæ and Odontaspididæ the vertebral centra differ from those of the preceding family in the diagonal lamellæ being greatly reduced or absent, and in the intermedialia being less completely calcified. The diagonal lamellæ are commonly regarded as absent, but in *Lamna* they can be recognised if searched for (fig. 9, B), and in *Cetorhinus* (fig. 14, A, p. 361) there are calcified structures which may have the same value, although they are rather too irregular to enable one to be certain; but it is extremely doubtful whether the isolated calcifications marked *i* in the diagonally placed cartilages of *Cetorhinus* are parts of the diagonal lamellæ. These "islands" are probably calcifications in the arch-cartilages themselves, and not in sheath-cartilage bulging into the arch-cartilages.

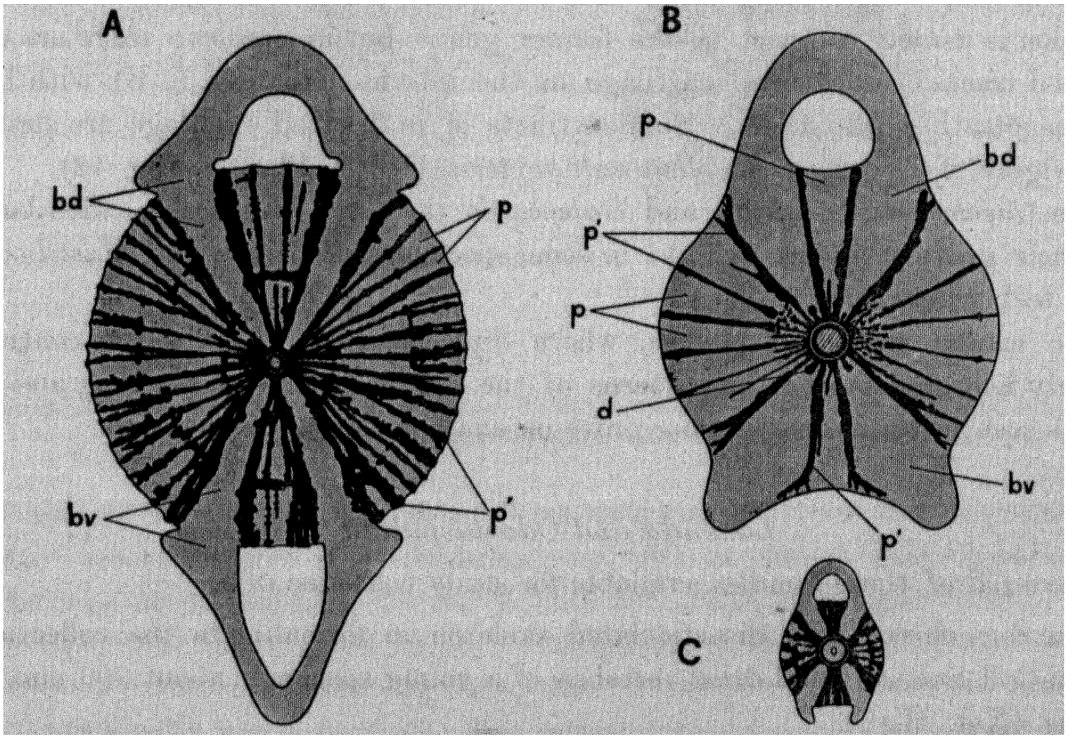


FIG. 9.—A, *Carcharodon rondeletii*, vertebra from anterior caudal region, × 0·75. B, *Lamna cornubica*, vertebra from cloacal region, nat. size. C, *Lamna cornubica*, half-grown, 920 mm. long, vertebra from cloacal region, nat. size. (For explanation of the lettering, see p. 404.)

The sharks of these families would seem to have a minimum of sheath-cartilage in the composition of their vertebræ. Remnants of the membrana elastica externa were found in *Odontaspis* alone of the fishes examined; although the young *Lamna* whose vertebra is represented in fig. 9, C, is a fish of about the same size, the search

for the membrane proved unsuccessful. In *Odontaspis* the remains of the membrane occur at the innermost end of each of the diagonal cartilages, and the line probably continues round in a circle passing through the inner ends of the four intermedialia, which are continuous with the investing layer of the double cone. HASSE's figure of an embryo *Lamna* (**15**, D, Plate 28, fig. 5) shows that the investing layer develops early. The figure is not clear, but it would seem that the eight structures that project outward from the double cone are calcified parts of the already developing intermedialia, the middle part of each of which is uncalcified, as, for instance, in an adult *Scapanorhynchus* (fig. 11, B, p. 358). HASSE does not show the membrana elastica externa, although it would be almost certainly recognisable in such a vertebra as he figures.

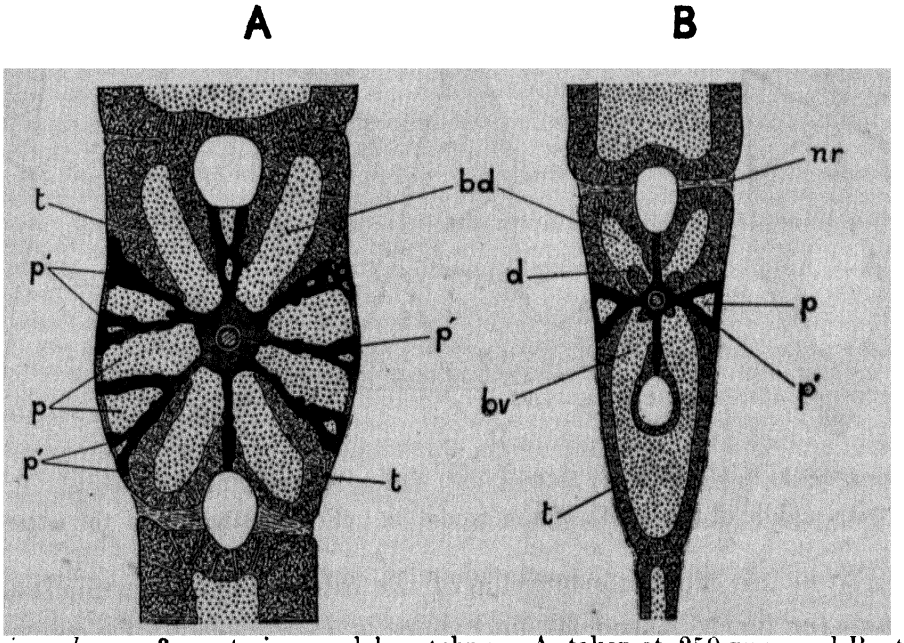


FIG. 10.—*Alopias vulpes*, × 3, posterior caudal vertebræ. A, taken at 250 mm., and B, at 120 mm. from the tip of the caudal fin. (For explanation of the lettering, see p. 404.)

The present investigation tends to confirm the observation of KOLLIKER (**20**, p. 75) that whereas in the Carchariidæ the inner ends of the intermedialia are regularly separate from the double cone in a section of the centrum taken midway between its anterior and posterior ends, in the families now under consideration the intermedialia are in adult fishes, and sometimes in young, confluent with the investing layer that surrounds the double cone. Each intermediale is, except in young *Cetorhinus* (fig. 13, A, p. 360), calcified on the two wedge-faces, *i.e.*, those faces which are in contact with arch-cartilage (fig. 11, A, p' and p''), and whereas the remainder may persist as uncalcified cartilage (fig. 11, p), it more usually has three or more radiating lamellæ within it (fig. 9, A and B). The intermedialia of *Cetorhinus* are exceptional in that in the young (fig. 13, A) the calcified lamellæ are all concentric but the wedge-faces calcify later, and radiating lamellæ are developed external to the earlier concentric ones (fig. 14, A).

The figures of adult *Lamna* vertebræ given by KÖLLIKER (**20**, Plate 13, fig. 3), HASSE (**15**, D, Plate 28, fig. 6), and GOODRICH* agree with that here given (fig. 9, B) in all essential features. The internal parts tend to become more irregular as age advances, and there may be a certain amount of absorption of the early deposits; the total number of calcified lamellæ in the lateral intermedialia varies from four to seven, and the uncalcified parts are fairly bulky.

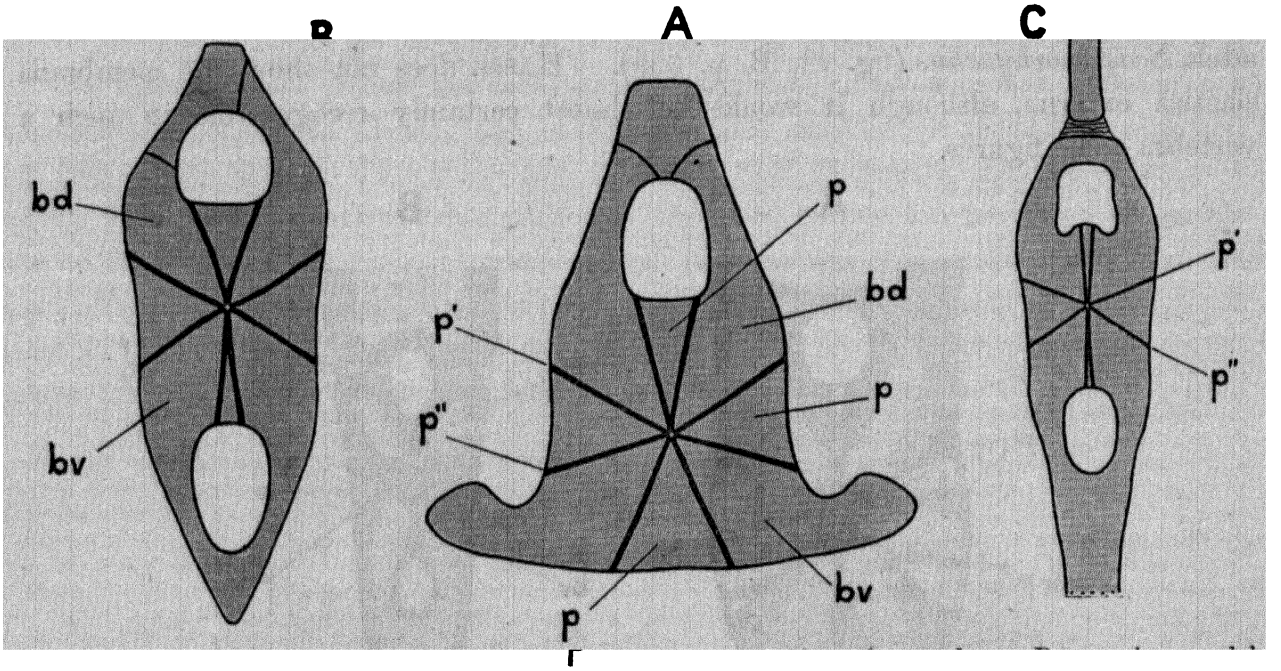


FIG. 11.—*Scapanorhynchus* (*Mitsukurina*) *owstoni*, nat. size. A, mid-trunk vertebra. B, anterior caudal vertebra. C, from middle of the length of the caudal fin. (For explanation of the lettering, see p. 404.)

In *Carcharodon* the radiating lamellæ of the intermedialia are much more numerous than in *Lamna* (*cf.* fig. 9, A and B), and there is a suggestion of a concentric arrangement, which is more or less apparent throughout the two families, and is not confined to *Cetorhinus* (KOLLIKER, **20**, p. 72). Vertebræ of *Carcharodon* have been previously figured by KOLLIKER (**20**, Plate 14, fig. 9), HASSE (**15**, D, Plate 30, fig. 30), and PARKER.† Those figured by PARKER are from his specimen A, a fish 10 feet long; that now figured is apparently from his specimen C, a fish 19 feet long.

The vertebræ of *Oxyrhina*, according to REGAN (**29**, p. 734), have no laminar structure, and exhibit a compact calcification like that of *Rhynchobatus*, but this is not in accord with the observations of KOLLIKER (**20**, Plate 13, fig. 5) and HASSE (**15**, D, Plate 31, fig. 41), who in their figures represent distinct radial lamellæ.

While in the majority of the sharks now under consideration there are no superficial calcifications in the arch cartilages, it is to be noted that in the hindermost caudal vertebræ of *Alopias* the tesseræ are strongly developed (fig. 10, p. 357). Two tails of *Alopias vulpes* were available for study. The vertebræ at the root of the

* 'Treatise on Zoology,' E. Ray Lankester, Part IX fasc. 1, London, 1909, p. 136, fig. 94, B.

† 'Proc. Zool. Soc.,' 1887, Plate 6, figs. 13 and 14.

great caudal fin show no remarkable features; the radiating lamellæ in the lateral intermedialia are less numerous than those of *Carcharodon*, although more numerous than those of *Lamna*. HASSE'S figure of *Hopias* (15, D, Plate 29, fig. 17) shows the lamellæ thicker and fewer than those in the specimens now under consideration; his fish was a small one.

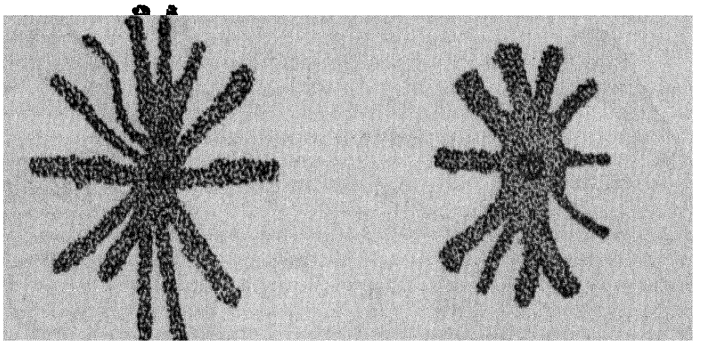


FIG. 12.—*Scapanorhynchus lewisi*, from the Upper Cretaceous of Mount Lebanon. Radiating lamellæ of two post-cloacal vertebræ, × 8.

In the part of the tail, however, about 250 mm. from the posterior extremity, the lamellæ of each lateral intermediale become reduced to a total of three or four (fig. 10, A), and in the region behind this the reduction is still greater. In fig. 10, B, are seen calcified nodules occupying the positions of the diagonal lamellæ, but these are not found in the more anterior vertebræ. What is of particular interest in these vertebræ is the manner in which the layers of tesseræ spread along the wedge-faces of the intermedialia, or, expressing it more correctly, the manner in which the outer parts of the intermedialia have expanded and spread over the tesseræ as the vertebræ grew in size; another instance of this is mentioned in the previous section in the case of *Galeus*. At a distance of 50 mm. from the posterior extremity of the excised vertebral column there are no secondary calcifications, only a typical double cone; and at 30 mm. from the extremity each centrum has two small, widely separated cones, with a rodlike granular or uncalcified part between (*cf. Raia*, pp. 318, 387).

In *Scapanorhynchus* it is only the wedge-faces of the intermedialia that calcify (fig. 11, p' and p''); the middle part of each (p), in formalin-preserved material, consists of a whitish-grey fibro-cartilage of cloudy appearance, in marked contrast with the pale blue hyaline cartilage of the arch-bases. The middle part of the double is very small, and the inner parts of the radiating calcified lamellæ are continuous with it. At the broad ends of the double cone the eight lamellæ are buttressed by partial lamellæ, which if more complete might extend so far as to appear in transverse sections through the middle of the length of the centrum, and thus cause such sections to resemble those of the more familiar sharks belonging to the family.

In REGAN'S classification (29) *Mitsukurina* is not recognised as generically distinct from the earlier extinct *Scapanorhynchus*, but it is to be noted that sections through the centra of *Scapanorhynchus lewisi* show radiating lamellæ more than eight in number

(fig. 12). My thanks are due to Dr. A. SMITH WOODWARD for having the two vertebræ cut in order that they might be compared with those of *Scapanorhynchus owstoni*. They are two consecutive vertebræ of the same fish. The dorsal part is uppermost in each figure.

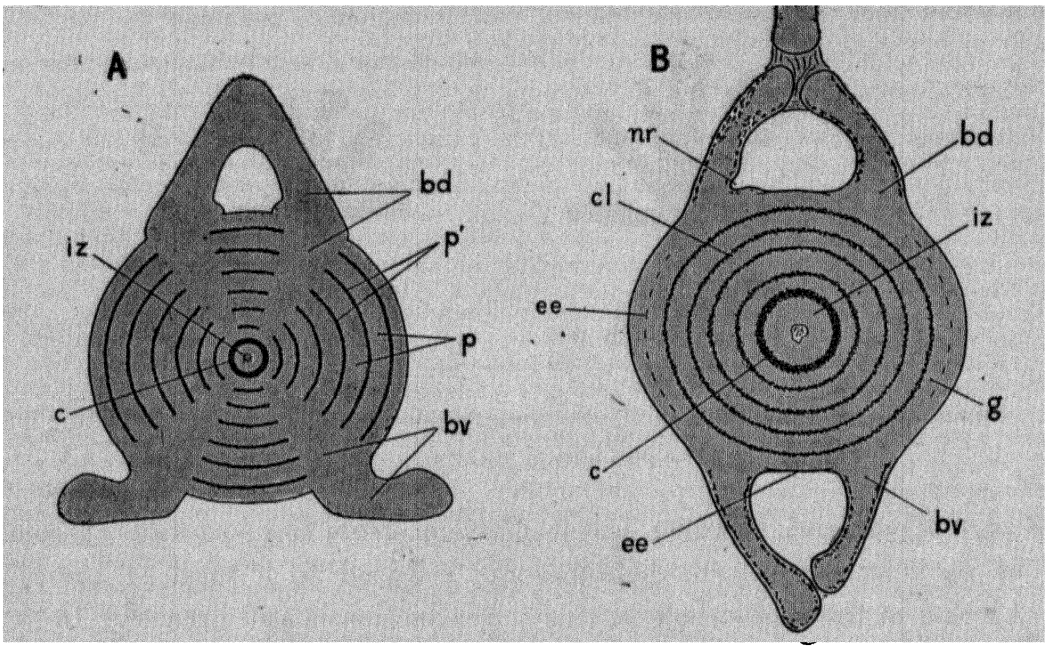


FIG. 13.—A, *Cetorhinus maximus*, young trunk vertebra, × 0·6. B, *Squatina squatina*, ripe embryo, 264 mm. long, caudal vertebra, from region of first dorsal fin, × 14. (For explanation of the lettering, see p. 404.)

It has already been pointed out that in young specimens of *Cetorhinus* the calcified lamellæ in the intermedialia are concentric. HASSE shows this in the caudal vertebræ (**15**, D, Plate 32, figs. 3 and 4); in fig. 13, A, above, it is demonstrated in a trunk vertebra. Allusion has also been made (p. 316) to the fact that for indicating taxonomic relationship the hind caudal vertebræ of the adult are in this genus more reliable than the trunk and anterior caudal vertebræ of the young; fig. 14, C, is recognisable as Lamnoid in character, but fig. 13, A, is peculiar. It will be noticed, further, how, in passing backward from the root of the caudal fin (fig. 14, A, B, C), the concentric laminæ dwindle and the radially disposed parts become relatively more important.

The wedge-faces, usually the first parts to undergo calcification, here calcify late. The concentric lamellæ of the young persist in the adult (fig. 14, A), and when isolated by removal of the cartilage by putrefactive maceration they are seen to be sieve-like (KOLLIKER, **20**, Plate 14, fig. 10), each perforation in the lamella being connected with a short calcified tube, which in life transmits a blood-vessel. These radiating tubes occur in great numbers, and give a characteristic appearance to the transverse section of the vertebra.

At the inner ends of the arch-cartilages are imperfectly defined calcifications that

may possibly be the equivalents of the diagonal rays of the Carchariidæ. In the middle of those parts of the arch cartilages that are situated between the intermedialia there are calcified islands (figs A and B, *i*) which vary considerably in size; in the vertebræ of the mid-trunk and branchial regions there are none in the basiventral cartilages; those in the basidorsals of the vertebræ of the branchial region are large and shaped like the letter T.

The vertebra shown as fig. 14, A, taken from the region just anterior to the caudal fin, is reproduced half the natural size. It is small, as the vertebræ of the basking shark go, for those of the precloacal region measure some 150 mm. across horizontally, while the maximum diameter of the open end of the double cone is 180 mm. The vertebræ of the branchial region are small, even smaller than that reproduced in fig. A.

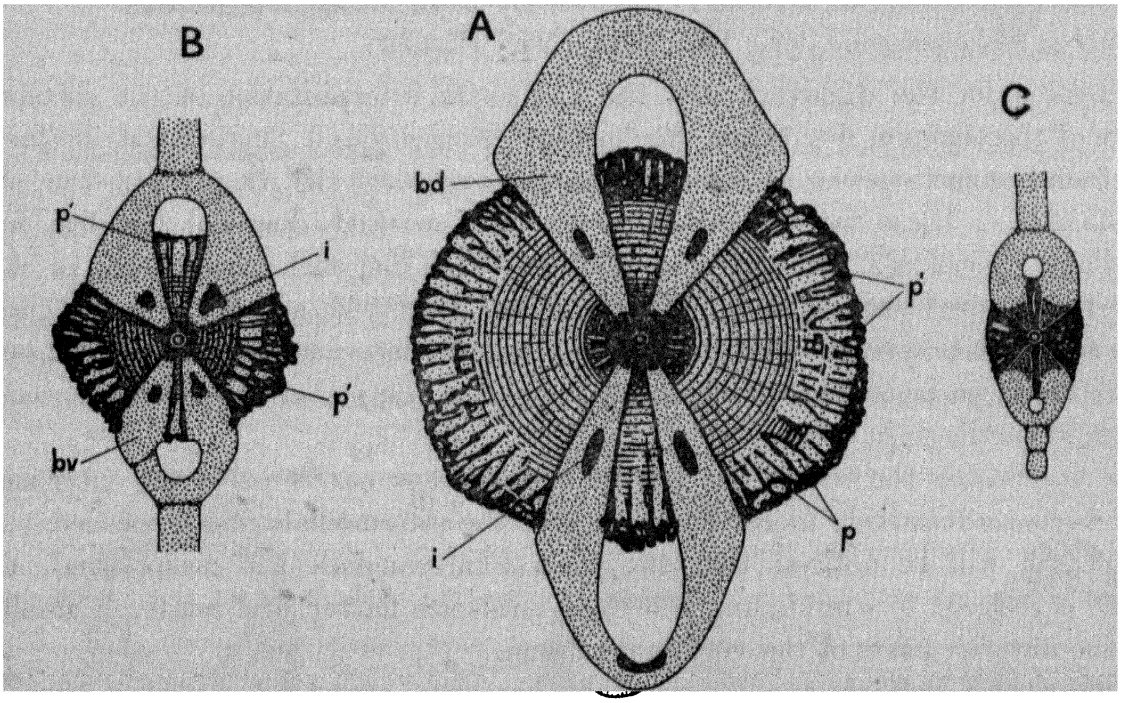


FIG. 14.—*Cetorhinus maximus*, ×0·5. A, caudal vertebra, from region just anterior to the caudal fin. B, vertebra about midway between those shown as figs. A and C. C, one of the hindermost vertebræ. (For explanation of the lettering, see p. 404.)

Judging from a plaster cast of a skeleton of *Cetorhinus* made at the Bergen Museum, and now exhibited at the Brit. Mus. (Nat. Hist.), caudal diplospondyly begins at the 30th vertebra. Counting each vertebral body in the caudal region as a centrum, without entering into the vexed question whether it is a centrum or a half-centrum, the vertebra drawn as fig. 14, A, would be the 52nd. Behind this there are another 54 centra.

In conclusion it may be pointed out, as an item of interest to the palæontologist, who frequently has to deal with isolated vertebral centra of Lamnid and Odontaspid sharks, that in the trunk region the ventral intermediale is wider than the dorsal, in

the cloacal region they are about equal, and as one passes backward along the tail the ventral intermediale becomes narrower and narrower. For the application of this knowledge, however, it is first necessary to know which is the dorsal side of the centrum, and in an isolated centrum this is not easy to ascertain.

Scyliorhinidæ.

The material available consisted of:—

Scyliorhinus canicula, six adults, purchased from the Marine Biol. Lab., Plymouth. Embryos, 5, 7, 10 and 55 mm. long, in the collection at the Royal Coll. Science.

Scyliorhinus stellaris, three adults; and one half-grown, 610 mm. long; purchased from the Marine Biol. Lab., Plymouth.

Scyliorhinus marmoratus, no locality, Brit. Mus. (Nat. Hist.).

Scyliorhinus analis, two, Sydney, presented by Prof. W. A. Haswell.

Pristiurus melanostomus, two, Brit. Mus. (Nat. Hist.).

To Hasse is due the discovery that the key to the interpretation of the vertebral structure of the common dog-fishes, *Scyliorhinus canicula* and *Scyliorhinus stellaris*, is to be found in such species as *maculatus* and *marmoratus* (**15**, D, pp. 242-253, and Plates 33-34). These latter species possess well-marked diagonal lamellæ, and intermedialia (periostale Strahlen und Keile). The diagonal lamellæ are as well developed as is the Carchariidæ (*cf.* fig. 15, A and B, p. 363, and fig. 8, p. 354), and bear the same relation to the sheath-cartilage; but the intermedialia are calcified only on the wedge-faces, as in *Scapanorhynchus* (fig. 11, p. 358), there remaining a wedge of unaltered cartilage in each.

As one approaches the end of the tail in *Scyliorhinus marmoratus* the outer-zone cartilage becomes relatively more abundant, and the intermedialia more reduced. In fig. 15, C, it will be noticed that the ventral intermediale has disappeared, the dorsal one is reduced to a minimum, and the laterals are flatter, and relatively smaller than in the anterior parts of the vertebral column.

In *Scyliorhinus stellaris* the intermedialia are more completely calcified than in *Scyliorhinus marmoratus*, and their edges grade off into the layer of tesseræ of the arch-cartilages. The trunk vertebra that is represented in fig. 16, B, was taken from an adult fish, and the limits of the outer-zone cartilage could not be distinguished; the position of the membrana elastica externa indicated in the figure was ascertained from a half-grown fish measuring 610 mm. in length. Hasse figures a caudal vertebra of a much younger fish, showing the membrana elastica externa still superficial in the four regions where the intermedialia will later be developed (**15**, D, Plate 34, fig. 17). A similar stage of development is seen in Goette's ripe embryo of *Scyliorhinus canicula* (**13**, Plate 29, fig. 18).

Goette, it is to be observed, dismisses the intermedialia of *Scyliorhinus* as mere "dünne Fortsetzungen" of the arch-cartilages, and "Verbindungsstücke der Wirbelbögen untereinander," and he is unable to agree with Kölliker as to their peri-

chondrial origin (**13**, p. 491); but he holds the same view with regard to the intermedial wedges of the Carchariidæ, in spite of the fact that his own figures (figs. 38 and 41) demonstrate the mode of origin that is claimed by KOLLIKER.

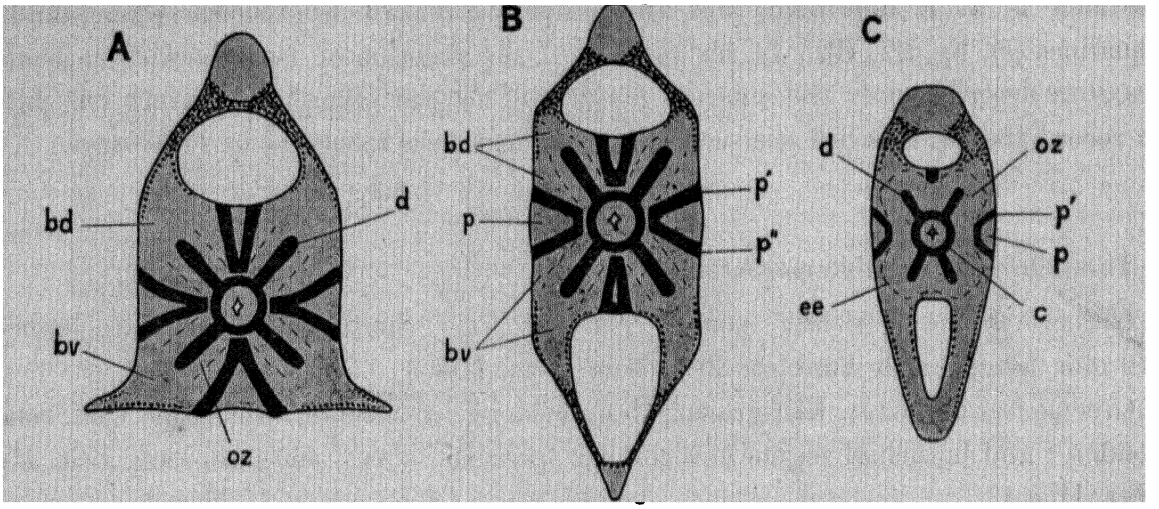


FIG. 15.—*Scyliorhinus maculatus*, ×8. A, vertebra from region of pectoral girdle. B, from region just anterior to the anal fin. C, from middle of the length of the caudal fin. (For explanation of the lettering, see p. 404.)

In a full-grown specimen of *Scyliorhinus canicula*, the calcification is less abundant than in a full-grown *Scyliorhinus stellaris*, and the relations are such as are shown in fig. 16, A. It will be noticed that there is a well-marked investing layer (*il*) around

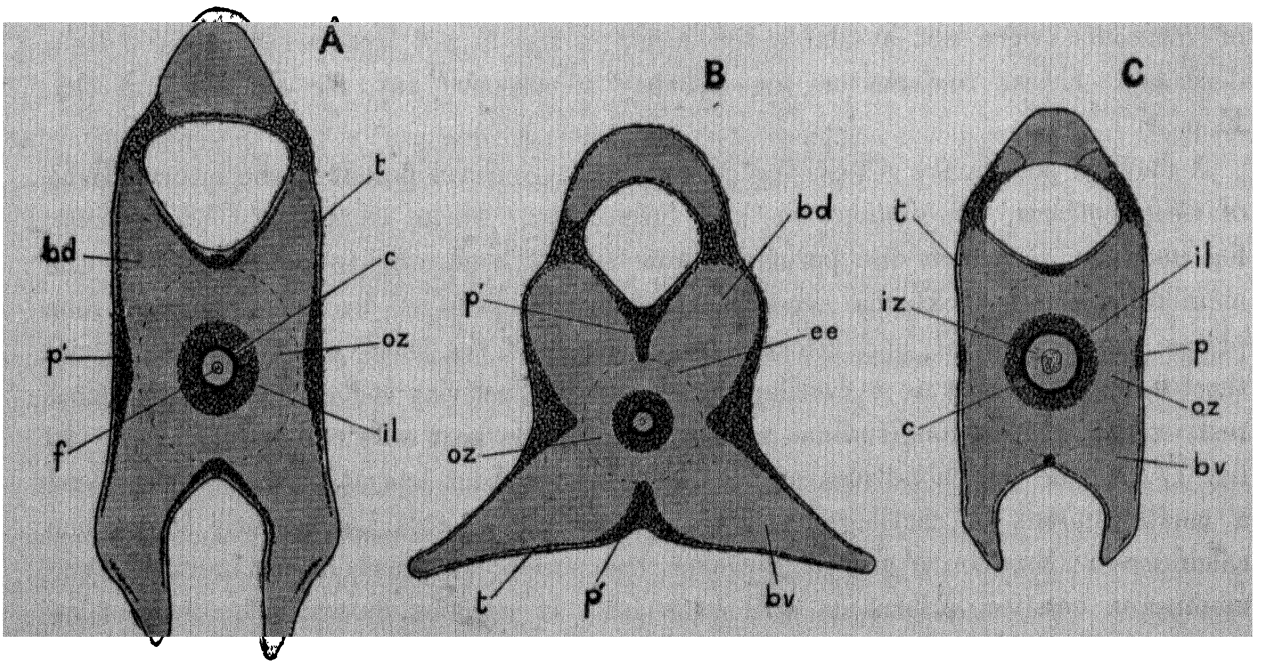


FIG. 16.—A, *Scyliorhinus canicula*, caudal vertebra, from region of first dorsal fin, ×8. B, *Scyliorhinus stellaris*, pre-cloacal vertebra, ×3·5. C, *Pristiurus melanostomus*, vertebra from region between first and second dorsal fins, ×9. (For explanation of the lettering, see p. 404.)

the double cone (*c*) in both species. The vertebræ of *Scyliorhinus analis* resemble those of *Scyliorhinus canicula*, not those of *Scyliorhinus marmoratus*.

In *Pristiurus melanostomus* (fig. 16, C) the degree of calcification of the intermedialia is at a minimum, and the general condition approaches that found in Squalines (*cf.* fig. 22, B). In his account of the skeleton of *Pseudotriacis microdon*, JACQUET describes only the external features of the vertebræ,* but REGAN has put it on record that in this fish the secondary calcification is feeble, as in *Pristiurus*.†

Orectolobidæ.

The material available consisted of—

Ginglymostoma cirratum, young, 325 mm. long, South Seas; and late embryo, 210 mm. long, South America, Brit. Mus. (Nat. Hist.).

Orectolobus barbatus, half-grown, 700 mm. long, in alcohol, Mus. Roy. Coll. Surg., London; and branchial region of a smaller specimen, about 460 mm. long, Brit. Mus. (Nat. Hist.).

Chiloscyllium plagiosum, adult, no locality; also young, 240 mm. long; also embryo, 120 mm. long, Brit. Mus. (Nat. Hist.).

Stegostoma tigrinum, half-grown, 660 mm. long, South India, vertebral column in alcohol, presented by J. HORNELL, Esq.; also embryo, 210 mm. long, N.W. Australia, Brit. Mus. (Nat. Hist.).

There is a fairly close agreement in the vertebral structure of *Chiloscyllium*, *Ginglymostoma*, and *Stegostoma*, whereas *Orectolobus* stands rather apart. Vertebræ of *Rhinodon* were not available for study; they do not seem ever to have been described. HASSE remarks on his inability to procure any for examination (**15**, E, p. 3).

A marked peculiarity is to be noted in the early development of the intermedialia of *Chiloscyllium*, *Ginglymostoma*, and *Stegostoma*. In an embryo of *Chiloscyllium*, for instance, in which the perichondrium is just beginning to become active, the membrana elastica externa occupies a superficial position in the customary four places, dorsal, ventral, and lateral; but, instead of the *whole* of each perichondrial tract becoming active as a cartilage-producer, the activity is for a considerable time restricted to its two longitudinal margins, the middle part only responding later. In fig. 17, E, the perichondrium of the right lateral tract has at p' and p'' produced a small amount of cartilage on its inner face, and this has already undergone calcification; between p' and p'', however, the perichondrium is as yet inert, and the membrana elastica externa is still external. It remains external for some time longer, during which the parts p' and p'' increase considerably (fig. F) by successive superficial increments, and the maintenance of its external position is to be attributed

* JACQUET, M., 'Bull. Mus. Océan. Monaco,' No. 36, 1905, pp. 28, eight Plates.

† REGAN, C. T., **29**, p. 745; and 'Ann. Mag. Nat. Hist.,' ser. 8, i, 1908, p. 464.

to a radial extension of the outer-zone cartilage. The actual distance, as measured in the slides, between the part marked *ee* and the nearest part of the cone (*c*), is greater in F than in E, but the inner parts of *p'* and *p''* are in F at the same distance from the double cone as in E.

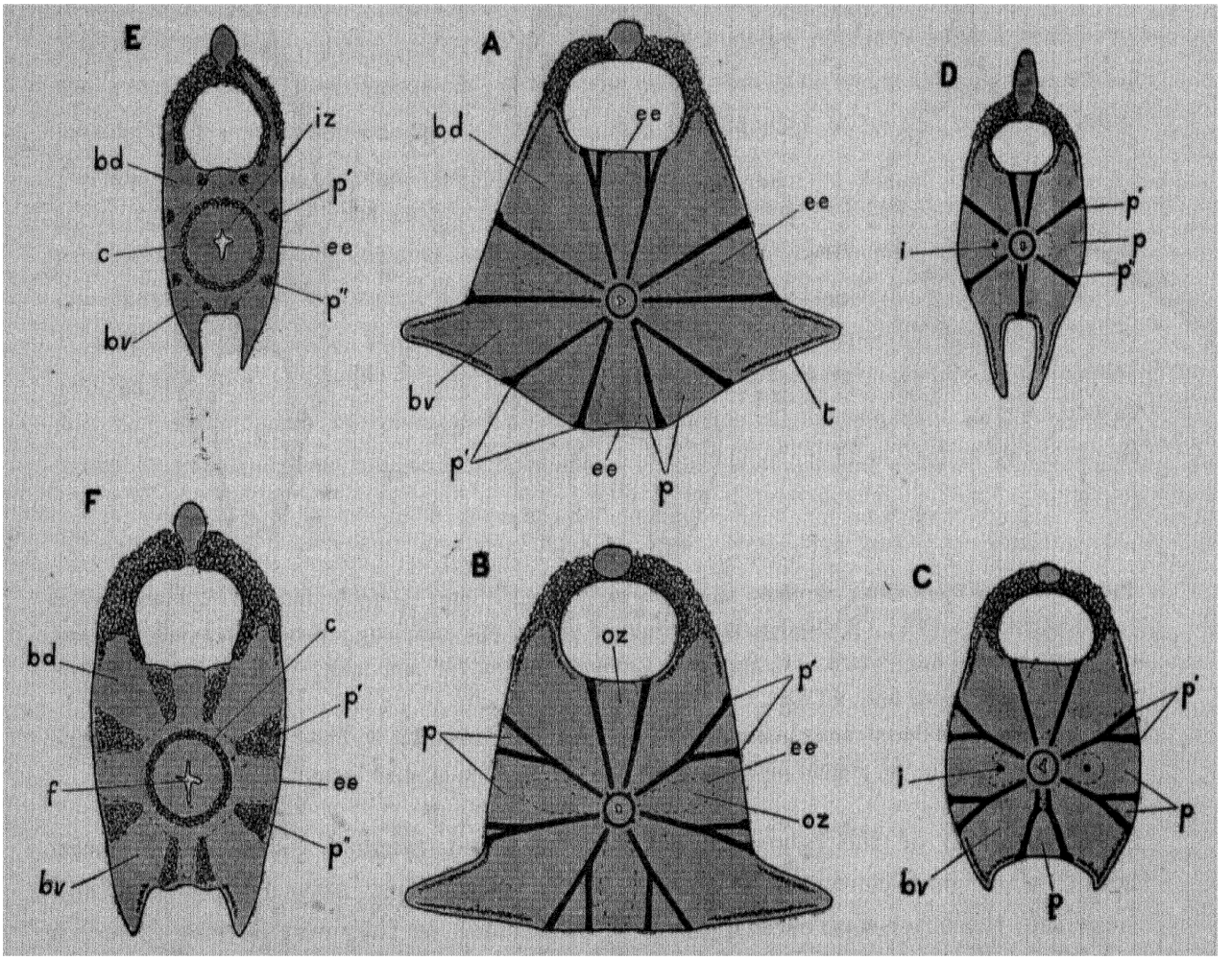


FIG. 17.—*Chlamydoselachus anguineus*. A, trunk vertebra of adult, from region of pectoral girdle, × 7. B, vertebra from region just anterior to base of pelvic fins, × 7. C, from region between first and second dorsal fins, × 7. D, from region of hind end of anal fin, × 7. E, vertebra from region just behind the first dorsal fin; embryo, 120 mm. long, × 17. F, vertebra from region just behind the first dorsal fin; embryo, 240 mm. long, × 17. (For explanation of the lettering, see p. 404.)

In fig. D, the magnification of which is less than that of figs. E and F, the parts *p'* and *p''* have grown into extensive radial lamellæ, but the outward spreading of the sheath-cartilage in the horizontal direction has ceased, and the membrana elastica externa is separated from the lateral surface of the centrum by a mass of uncalcified cartilage (*p*), produced by the part of the perichondrium which was at first inert. The same fate does not befall the outer-zone cartilage in the vertical direction; the upper tract continues to grow upward, and to maintain the membrana elastica externa in a superficial position, in all vertebræ (A–D); in the lower tract, however,

the activity of growth of the sheath-cartilage diminishes as one passes backward along the vertebral column (B, C).

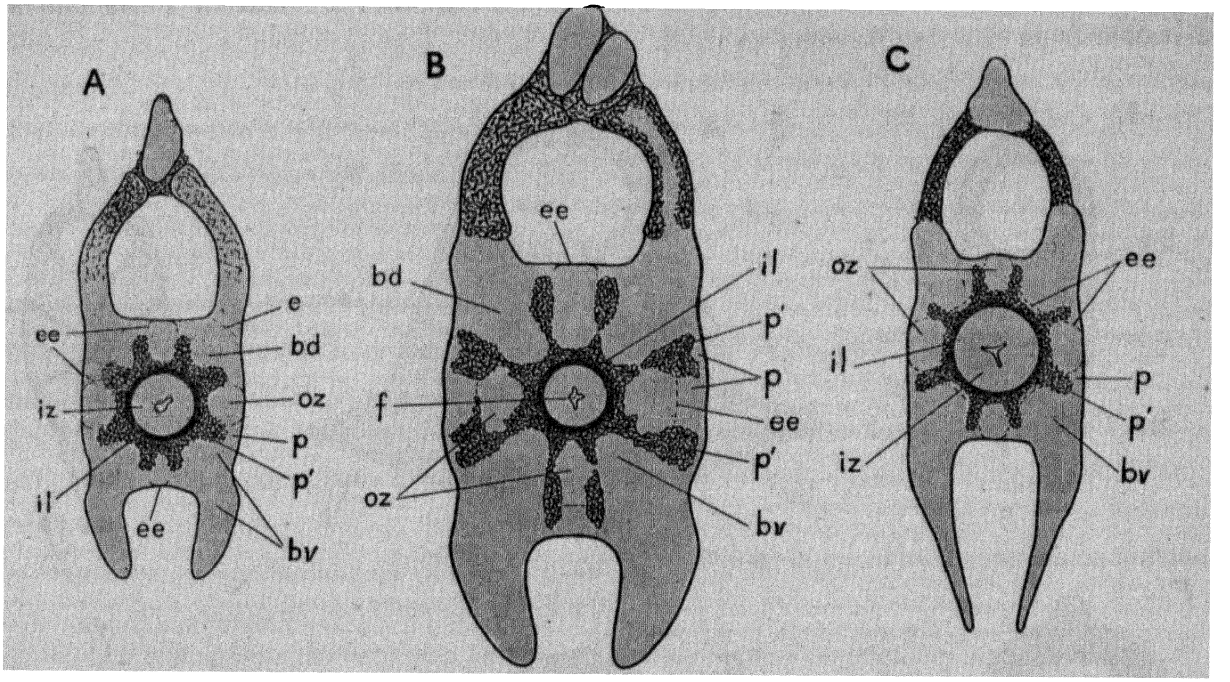


FIG. 18. A, *Ginglymostoma cirratum*, late embryo, 210 mm. long; vertebra between first and second dorsal fins, × 13·5. B, *Ginglymostoma cirratum*, young, 325 mm. long; vertebra between first and second dorsal fins, × 13·5. C, *Stegostoma tigrinum*, embryo, 210 mm. long; vertebra between pelvic and second dorsal fins, × 13·5.

e, position of membrana elastica externa according to HASSE, 15, D, Plate 26, fig. 19; *ee*, position as ascertained in the present investigation. (For explanation of other lettering, see p. 404.)

In vertebræ of the caudal region may be found calcified islands in the horizontal plane that contains the axis of the centrum; they may develop late, and lie within the outer-zone cartilage (fig. C, right side), or they may develop in such a manner as to check the outward bulging of the sheath-cartilage (figs. C and D, left side). Diagonal rays are not altogether wanting; traces of them are shown in fig. B.

The lateral calcified rays are simple in the anterior and posterior parts of the vertebral column, but forked in the middle parts (*cf.* A, D and B, C); the upper rays fork only in the branchial region (A); the lower rays are confluent in the hindermost vertebræ (D), simple in the anterior caudal (C), forked more anteriorly (B), and in the branchial region are V-shaped, rather than Y-shaped (A).

Ginglymostoma and *Stegostoma* differ from *Chiloscyllium* in the greater extent to which the investing layer is developed (*cf.* figs. 18 and 17); and, largely as a consequence of this, the innermost parts of the eight calcified lamellæ originate in sheath-cartilage (fig. 18, A and C). Further than this, the perichondrially produced cartilage undergoes calcification rather later, so that the outer ends of the calcified rays are relatively farther from the surface. The line of demarcation between the uncalcified perichondrial cartilage and the arch-cartilage is not so well

marked as in *Chiloscyllium*, the texture of the two cartilages being almost the same, whereas in *Chiloscyllium* the perichondrially produced cartilage is a small-celled cartilage; in fig. 18, A, the limit between the two cartilages follows roughly the indicating line that is drawn from the letter *p'*; the cartilage immediately above the line is new cartilage of perichondrial origin (*p*), that below the line is the original, although still expanding, arch-cartilage (*bc*). The stage of development shown by the young *Ginglymostoma* in fig. 18, B, is relatively later than that of the young *Chiloscyllium* represented in fig. 17, F, for the lateral parts of the membrana elastica externa are no longer superficial.

In fig. 18, B, it will be noticed that there is a constriction between the proximal and distal parts of the rays, or even a separation of the parts, which is of interest in connection with KOLLIKER'S figure of *Ginglymostoma* (**20**, Plate 13, fig. 1, 1), in which the detachment is still more pronounced. Vertebræ of full-grown *Ginglystoma* were not available for study. According to HASSE'S figure (**15**, D, Plate 26, fig. 21), the caudal vertebræ closely resemble those of *Stegostoma*; in the anterior trunk region the rays branch to a very great extent (*ibid.*, Plate 26, fig. 24).

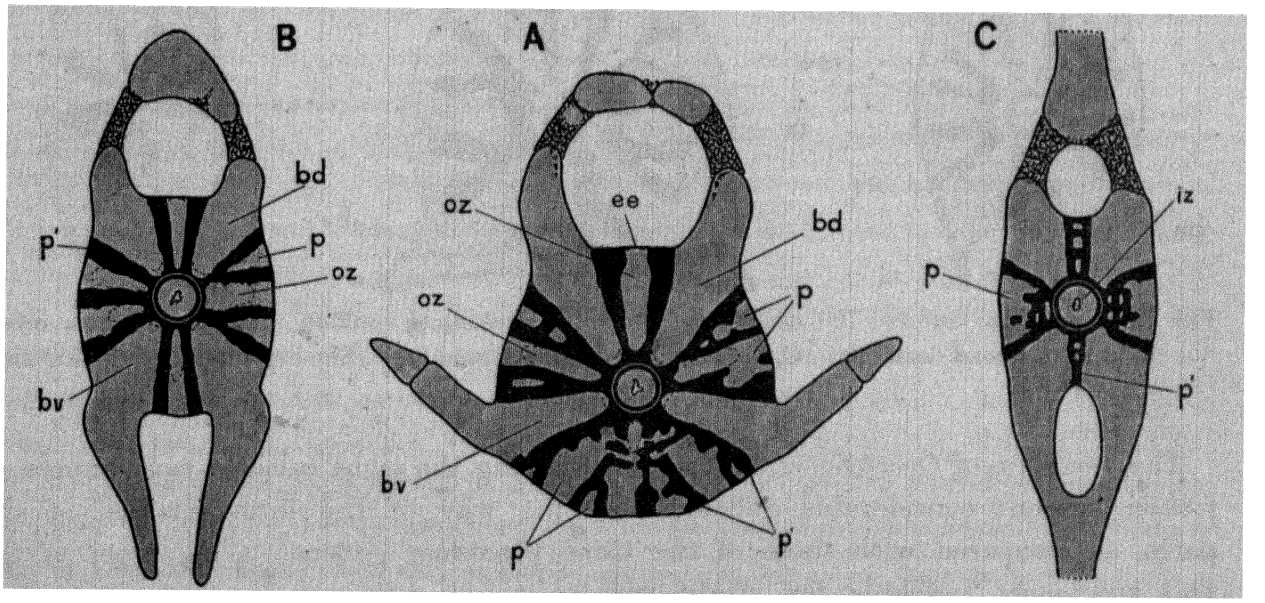


FIG. 19.—*Stegostoma tigrinum*, 660 mm. long, × 6. A, vertebra from region of pectoral girdle. B, vertebra from post-cloacal region; for younger stage, see fig. 18, C. C, vertebra from region half way along the caudal fin. (For explanation of the lettering, see p. 404.)

HASSE'S interpretation of the parts of the vertebra (**15**, D, Plate 26, fig. 19) differs materially from that here given, for he represents the membrana elastica externa as following a more or less circular course, as shown at *e* in fig. 18, A, of this paper. Similarly, in *Stegostoma*, the particular tract of cartilage which, on the right side of fig. 19, B, of this paper is marked *p*, to signify cartilage of the intermediale, is marked by HASSE, on the left side of his fig. 3, Plate 25, with the letter *a*, to denote outer-zone cartilage. It is strange that HASSE, while

regarding the calcified lamellæ of *Chiloscyllium* (**15**, D, Plate 40, figs. 4 and 5) as parts of the Periostale Keile, did not do so in *Stegostoma* and *Ginglymostoma* (*ibid.*, p. 197, para. 2; p. 202, para. 5).

Comparison of figs. 19 and 17 shows that in a well-grown, though not adult *Stegostoma* the rays, while more irregular, have the same general arrangement as in *Chiloscyllium*. Remnants of the membrana elastica externa can be seen in all except the hindermost vertebræ. The normal condition in the anterior caudal vertebræ is evidently one in which the lateral rays are two Y-shaped rays (**15**, D, Plate 25, fig. 3), but in the material examined it happened, curiously enough, that three successive vertebræ possessed three separate rays on one side (fig. 19, B), with outgrowths of outer-zone cartilage between them.

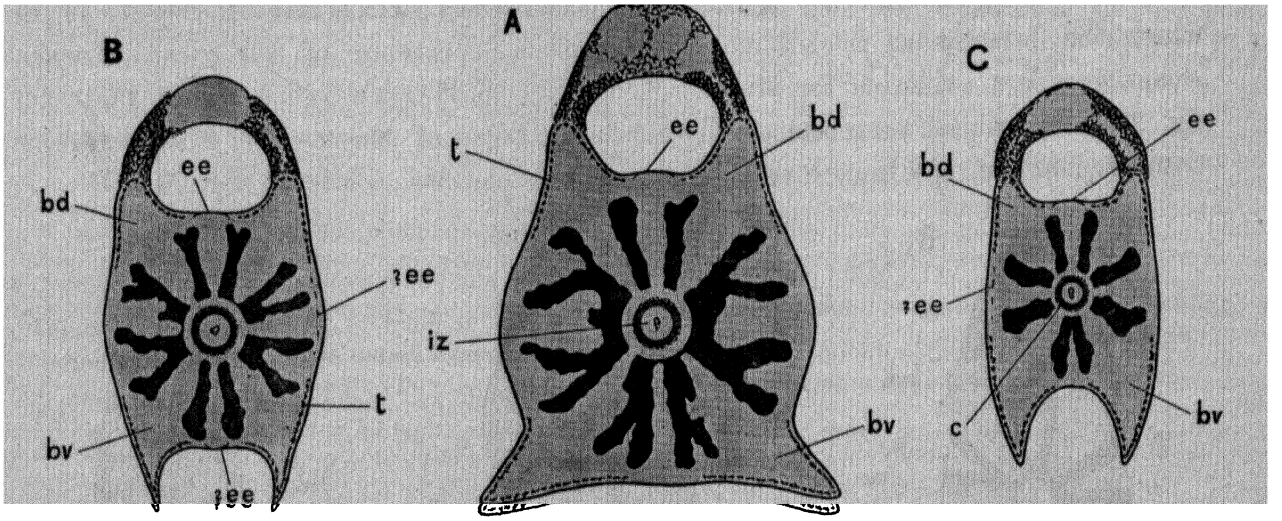


FIG. 20.—*Orectolobus barbatus*, 700 mm. long, × 4·6. A, precloacal vertebra. B, vertebra from region in front of second dorsal fin. C, vertebra from region of anal fin. (For explanation of the lettering, see p. 404.)

The vertebræ of *Orectolobus* although possessing the eight calcified rays, simple or forked, present considerable difficulties in the way of the interpretation of the parts, as compared with those of the three preceding genera. The larger of the two specimens available for study was only half-grown, being not more than 700 mm. long, but its vertebræ appear to be less developed, although slightly larger, than those figured by HASSE (**15**, D, Plate 25, figs. 4–6). In fig. 20, the calcified rays are seen not to touch the double cone, nor do they reach the surface, yet they are represented as extending from double cone to surface in HASSE's fig. 6. HUBRECHT gives a figure* just clear enough to be recognisable as an Orectolobid vertebra, and he states that the radiating calcified lamellæ are "allenthalben von der Elastica externa umschlossen," which is the same view as that of HASSE (**15**, D, p. 199). In my own sections the only unmistakable remnants of the membrana elastica externa are those in the floor of the neural canal, but there are

* BRONN'S 'Klassen und Ordnungen des Thierreichs,' Bd. VI, Abth. 1, Lief. 3, 1878, Plate 10, fig. 13.

doubtful remnants in the roof of the hæmal canal of the caudal vertebræ, and others at the side of the centrum (fig. 20, B and C, ? *e e*), which, if verified by recourse to more suitable material, would necessitate an admission that the calcified rays are not perichondrial in origin, and thus differ from those of *Chiloscyllium* and *Stegostoma*. It is difficult without further evidence to accept this position, but it is to be noted that in some parts, as at *t* in fig. B, there are tesseræ immediately external to one of the calcified rays, which could scarcely happen if the ray were growing outwardly by additions from the external perichondrium. Further research on this genus is clearly in demand.

In the sharks of the family Orectolobidæ the supradorsal cartilages in certain parts of the vertebral column tend to run in double series, but not with any regularity (fig. 18, B; fig. 19, A; fig. 20, A; and HASSE, **15**, D, Plate 25, fig. 6; Plate 40, fig. 4).

Cestraciontidæ.

The material available consisted of:—

Cestracion philippi, three adults and one young, 260 mm. long, Brit. Mus. (Nat. Hist.): and caudal regions of three fishes, large, medium, and small; also a ripe embryo, 180 mm. long, Sydney, presented by Prof. W. A. HASWELL.

The characteristic structure of the caudal vertebræ of *Cestracion* is that displayed in figs. 21, A and C. The calcified rays as seen in the section are eight in number,* but one or more of them may be forked; they are approximately symmetrical in disposition, and the upper and lower pair are directed towards the inner edges of the basidorsal and basiventral cartilages respectively, while the laterals are directed towards the outer edges of those cartilages. The inner ends of the rays are from the first in contact with the double cone, which has a very thin investing layer, and the outer ends are in some cases strangely enlarged. None of the rays reach the surface; they all terminate well within the sheath-cartilage, the outer limit of which is indicated by the remnants of the membrana elastica externa that are visible even in the adult.

The vertebra shown at fig. 21, C, is from the same region of the tail as that shown at A, but is taken from a younger fish; the membrana elastica externa is superficial above, below, and at the sides of the centrum. In older vertebræ (*e.g.*, A) it is superficial above, but not in the other three regions, and here difficulties arise in regard to the interpretation of the parts that are immediately external to the membrane. As is shown on the right side of fig. A, the calcifications (? *p'*) may be separate from the tesseræ of the arch-cartilages (*t*), or they may be in continuous series with them, as shown below and on the left side, and there is nothing in the histological structure to enable one to decide whether there is in the parts in question a perichondrial activity, producing a feebly developed intermediale, or a

* 'Bei Cestracion . . . sind regelrecht acht Strahlen vorhanden,' **15**, A, p. 51, para. 2.

fusion of arch cartilages such as the continuity in the layer of tesseræ might suggest. In the trunk region there can scarcely be any doubt that the basidorsal and basiventral cartilages are continuous at the sides of the centrum (fig. 21, D), just as there is in the Batoid fishes (*cf.* A and B, fig. 4, p. 340). What adds to the difficulty in the way of interpretation is that in some of the more posterior caudal vertebræ (fig. 21, B), the calcification marked ?*p'*, or at least some part of it, is *internal* to the membrana elastica externa. In the hindermost vertebræ the calcified rays become fewer and more irregular (fig. B).

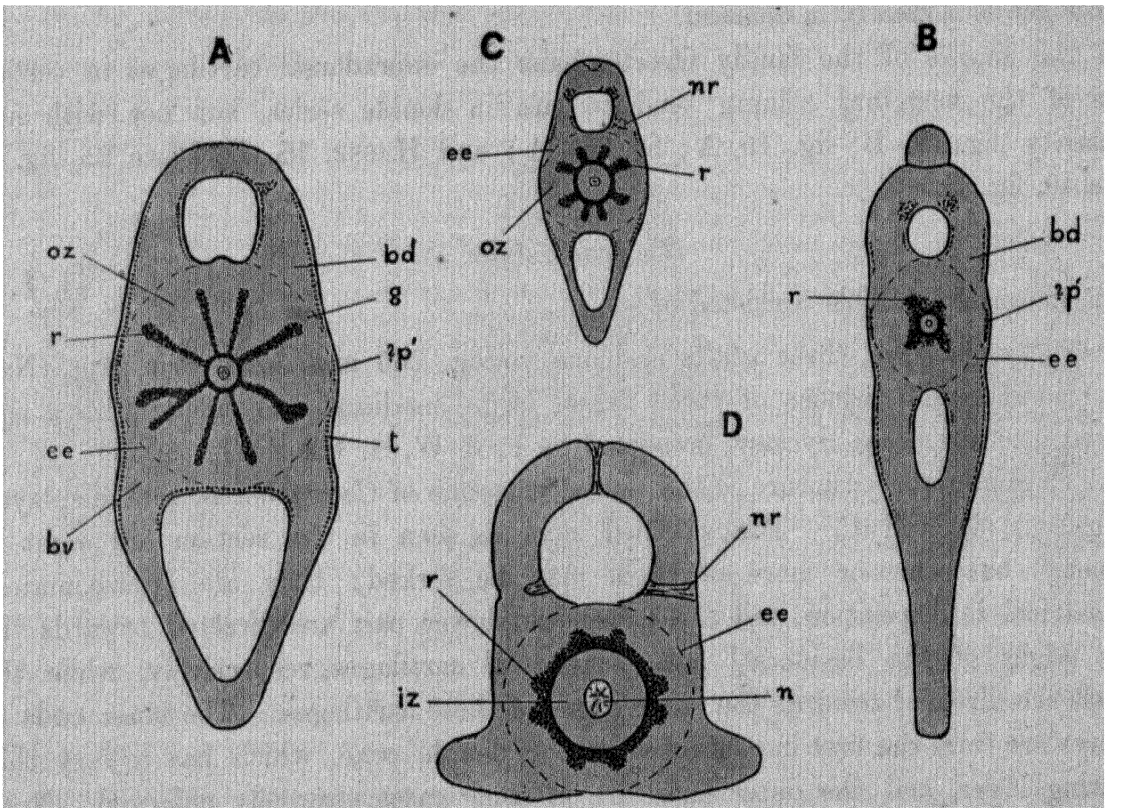


FIG. 21. *Centracion philippi*. A, caudal vertebra from region of second dorsal fin, × 3. B, a more posterior vertebra of the same fish, from region of hinder part of front lobe of caudal fin, × 3. C, vertebra from region of second dorsal fin of a half-grown fish, × 3. D, vertebra from branchial region of a ripe embryo, 180 mm. long, × 9. (For explanation of the lettering, see p. 404.)

In the trunk region of the vertebral column eight primary rays can be distinguished in early stages of development (fig. 21, D), but in adults the rays are more numerous, and they are so evenly spaced around the double cone that it is not possible to explain how the condition can have arisen by a process of forking and branching of the eight rays. In fig. 1, A, p. 340, for instance, while the upper and lower pairs can be identified, it is to be noted that there are three main lateral rays, instead of two, on each side.

HASSE does not give a really characteristic figure of the vertebra of *Cestracion*, but his interpretation of the calcified rays as belonging to the outer-zone cartilage is in agreement with that here given (**15**, D, Plate 24). The figures given by

DANIEL ('Journ. Morph.,' xxvi, 1915, Plate 6, figs. 20 and 23) show that the vertebral structure of *Cestracion francisci* agrees with that of *Cestracion philippi*; there are eight radiating lamellæ in the caudal vertebra that he figures, and twelve in a vertebra from the branchial region.

Squalinæ.

The material available consisted of:—

Echinorhinus spinosus, vertebral column in formalin solution, Brit. Mus. (Nat. Hist.); also selected vertebræ in alcohol, in the collection at the Royal Coll. Science.

Oxynotus centrina, two, Brit. Mus. (Nat. Hist.).

Spinax niger, two, Brit. Mus. (Nat. Hist.).

Squalus acanthias, two adults, purchased, Grimsby; three adults, purchased, Folkestone; one adult, purchased, Plymouth; one embryo, 60 mm. long, and two late embryos, 210 mm. long, in the collection at the Royal Coll. Science.

Centrophorus calceus, vertebral column of adult in alcohol, presented by Prof. W. N. F. WOODLAND; one young, 250 mm. long, Brit. Mus. (Nat. Hist.).

Centrophorus squamosus, vertebral column in alcohol, Brit. Mus. (Nat. Hist.).

Scymnorhinus lichia, vertebral columns of two specimens, in formalin solution, Brit. Mus. (Nat. Hist.).

Somniosus microcephalus, vertebræ in alcohol, Brit. Mus. (Nat. Hist.).

Somniosus rostratus, skeleton in alcohol, Brit. Mus. (Nat. Hist.).

There is a striking uniformity in the essential features of the vertebræ of the Squaline fishes, not only as between different fishes, but also as between the caudal and trunk vertebræ of the same individual fishes. The double cone may be either long or comparatively short (fig. 2, p. 321), and it is fairly wide open in the middle; a moderately thick investing layer is present in most cases. Remnants of the membrana elastica externa are usually recognisable in the vertebræ of adult fishes (fig. 22, p. 372), and these serve to show that there is a large proportion of outer-zone cartilage entering into the composition of the definitive centrum.

The outer crust on the arches commonly takes the form of a continuous compact layer rather than a series of separable tesseræ. The crust is thick in the case of *Scymnorhinus*, particularly at the anterior and posterior ends of the centrum, where it is continuous with the investing layer of the double cone; in preparing the double cone of *Scymnorhinus* represented in fig. 2, A, the end portions of the crust were removed by means of a file.

In the trunk vertebræ of the Squalinæ the basiventral cartilages are set fairly high up, and there is an obvious fusion of the basidorsal and basiventral cartilages on each side of the centrum (fig. 22, A and C); the basiventral may in some cases be segmented into an upper part which bears the rib, and a lower part, termed the aortic support (*Somniosus*, SCHAUINSLAND, **30**, p. 411, fig. 213, C).

In caudal vertebræ there is a lateral tract which I regard as of perichondrial origin (fig. 22, B, *p*); though mainly cartilaginous, it may be partly calcified.* On the inner side of this, but external to the membrana elastica externa, is a calcified layer that is continuous above and below with the general external crust of the arch-cartilages; the origin of this part is doubtful. In the floor of the neural canal and in the roof of the haemal canal there is a continuous curved calcified layer, but the middle parts of this lie on the inner side of the membrana elastica externa, and thus occur in the sheath-cartilage.

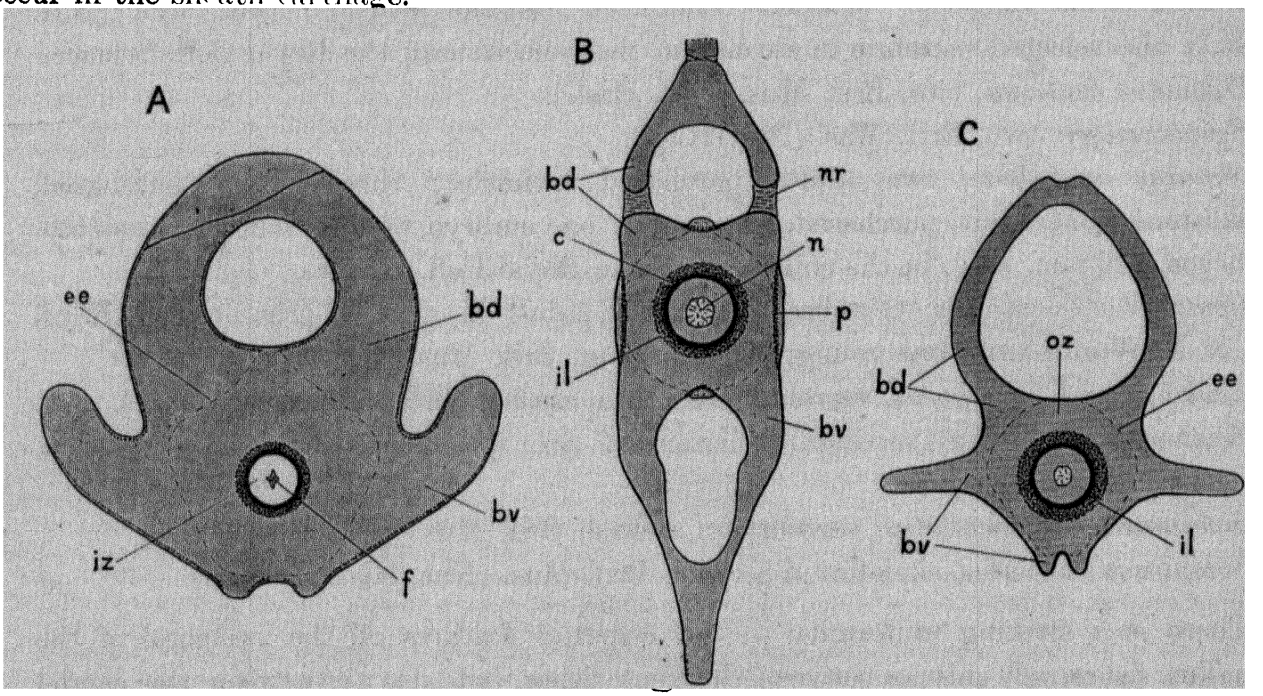


FIG. 22.—A, *Oxynotus centrina*, trunk vertebra, × 3. B, *Squalus acanthias*, caudal vertebra, × 4. C, *Spinax niger*, trunk vertebra, × 10. (For explanation of the lettering, see p. 404.) (The part marked *iz* in fig. A should have been shaded, as in B and C.)

Sections through the vertebræ of the ripe embryo of *Squalus acanthias* show an early differentiation of the sheath-cartilage into the three zones, with incipient calcification of the middle zone; the notochord is as yet scarcely constricted by the growing inner-zone cartilage; the membrana elastica externa is still external at the sides of the centrum, and above and below. No illustration is here given, since the features are exactly those shown in the figures of GOETTE (**13**, Plate 30, fig. 30) and HASSE (**15**, B, Plate 11, fig. 19). In the embryo of a length of 60 mm. the differentiation of the sheath into the three zones has not yet taken place.

Except in the terminal caudal vertebræ, there are in *Somniosus microcephalus* and *Echinorhinus spinosus* no calcified structures. As in the case of *Hexanchus*, the intervertebral ligaments are long and the "centra" are represented by thick septa, perforated in the middle by the funicle (fig. 23). The vertebral column in these fishes

* It is not shown in HASSE's figure, **15**, B, Plate 12, fig. 20.

is clearly degenerate, and it is of particular interest to recall the fact that in *Somniosus rostratus* the vertebræ are not reduced in number as they are in *Somniosus microcephalus*, and they are typically Squaline in character, the double cones being well developed, although the external crust of the arches is thin or wanting.* Seeing that HASSE's figures of *Echinorhinus* (15, B, Plate 9) are drawn of the natural size, it is clear that his specimen was not more than half-grown.

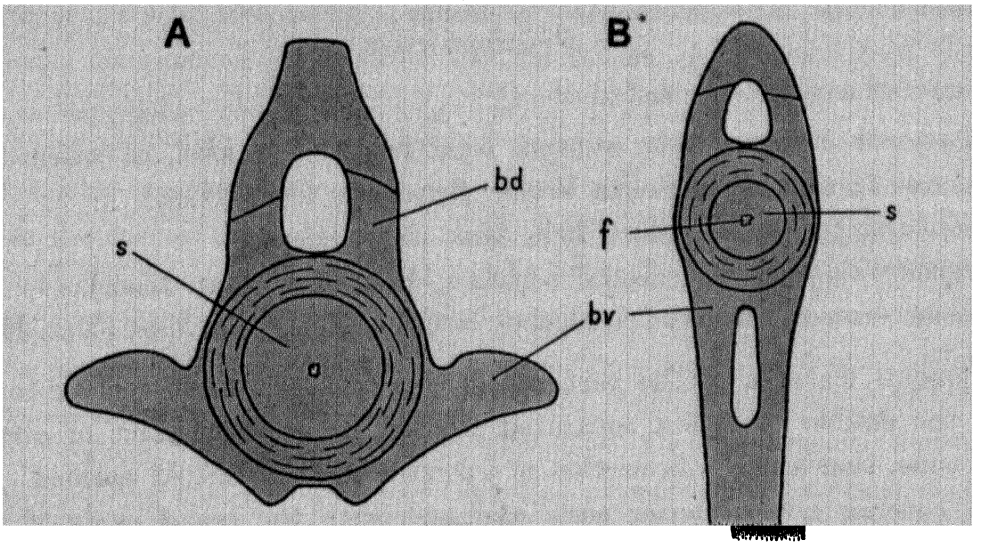


FIG. 23.—*Echinorhinus spinosus*, nat. size. No parts are calcified. A, mid-trunk vertebra. B, vertebra from region between second dorsal and caudal fins. (For explanation of the lettering, see p. 404.)

The terminal caudal vertebræ of *Echinorhinus* were not available for study; in those of *Somniosus microcephalus*, as mentioned on p. 318, there are reduced vertebral structures, namely, external patches of irregular granular calcification (HASSE, 15, B, Plate 8, fig. 5; also HELBING,* p. 446 [112]) and internal granular calcified tubes or rods with dilated ends, representing degenerate double cones.

No specimen of *Centroscyllium* was available, but KOLLIKER has remarked (20, p. 55) that the vertebræ resemble those of *Squalus* (*Acanthias*). It will be observed that KÖLLIKER regards the thin tract that lies external to the membrana elastica externa at the side of the centrum as due to a fusion of the arch cartilages, such as occurs in the trunk region, and not as a new structure of perichondrial origin. One can see, he writes, "dass die Wirbelkörper eine zarte Belegung von den knorpeligen Bogen besitzen und dass die oberflächliche Verkalkung derselben diesem der Chordascheide fremden Knorpel angehört." HASSE's figures of *Centroscyllium* (15, B, Plate 10) show that the vertebræ present no special features.

HASSE records that the vertebræ of *Isistius* agree with those of *Somniosus rostratus* and *Scymnorhinus*, but approach the latter more than the former (15, E, p. 8). WOODLAND's figures of the vertebræ of *Centrophorus* call for no special comment

* HELBING, 'Nova Acta Abh. kaiserl Leop Carol. Akad.,' lxxxii, 1, Halle, 1904, pp. 138–448 (104–114) also HASSE, 15, B, Plate 8, figs. 7–12.

('Proc. Zool. Soc.,' 1906 (1907), Plate 58, figs. 7 and 8); neither do HASSE'S figures (**15**, B, Plate 11).

Whereas caudal diplospondyly prevails in Elasmobranchs generally, it has been held that *Scymnorhinus* is exceptional (**6**, p. 195). The specimen of *Scymnorhinus* examined in the course of this inquiry exhibits the same diplospondyly as the other members of the family; so also does the figure given by MAYER (**24**, Plate 18, fig. 9).

Pristiophorinæ.

The material available consisted of—

Pristiophorus cirratus, late embryo, total length 300 mm., of which the rostral saw measures 75 mm., New South Wales, Brit. Mus. (Nat. Hist.).

Pristiophorus japonicus, adult, Brit. Mus. (Nat. Hist.).

Pristiophorus nudipinnis, caudal vertebræ, Brit. Mus. (Nat. Hist.).

Pliotrema warreni, skeleton in alcohol, South Africa, Brit. Mus. (Nat. Hist.).

The general features of the vertebræ of *Pristiophorus* are displayed in fig. 24, A. Around the double cone, but separated from it by a broad tract of cartilage, is a calcified tube, that appears in section as a square with truncated corners. This tube is at its anterior and posterior ends confluent with the broad parts of the double cone; it is not to be regarded as a wide-standing investing layer, for a thin investing layer is present also, situated immediately external to the double cone. In HASSE'S figure of the vertebra of the same species (*Pristiophorus japonicus*, **15**, C, Plate 13, fig. 5) the lateral parts of the main calcified "ring" are incomplete, but his figure of *Pristiophorus cirratus* (*ibid.*, fig. 4) agrees with fig. 24, A, of the present paper.

There are four small superficial calcifications of perichondrial origin (p') above, below, and at the sides of the centrum. Remnants of the membrana elastica externa are recognisable in the adult, and, in *Pristiophorus japonicus*, the course taken by the membrane, as seen in a transverse section, is one that passes through the inner part of an intermedial calcification, then across the corner of the main ring, and then through the inner part of the next intermediale. Thus it will be seen that the outer-zone cartilage bulges out horizontally and vertically, and that the calcified ring occurs partly in sheath-cartilage and partly in arch-cartilage. That too much importance should not be attached to this, however, is evident from the fact that, in caudal vertebræ of *Pristiophorus nudipinnis*,* the course of the membrane, while agreeing, in so far as its relations to the intermedial calcification are concerned, pursues a less sinuous line, and remains external to the main calcified ring. The ring is consequently, in this latter species, a structure that belongs solely to the sheath-cartilage.

In *Pliotrema warreni* (fig. 24, B) the main calcified ring is thin, and with a

* Only posterior caudal vertebræ, from the base of the caudal fin, were available for study.

tendency to be discontinuous; from it there project eight strongly marked rays, that broaden externally, and fail to reach the surface. The upper and the lateral calcified intermedialia are wanting, and the lower one is small. The membrana elastica externa follows the same course as in *Pristiophorus japonicus* (fig. 24, A), the parts in the diagonal positions being internal to the calcified ring. It passes through the inner part of the lower intermedial calcification, and just reaches the surface of the centrum above, at the bottom of a slight depression between the two basidorsal cartilages. At the sides of the centrum the membrane is separated from the surface by a tract of cartilage, which, by analogy with *Pristiophorus*, would be taken to be intermedial cartilage, but, in the absence of embryological evidence the point cannot be decided, for the texture of the cartilage affords no indication of its mode of origin. Similarly, in the case of the outer parts of the eight calcified rays, a difficulty arises; the membrana elastica externa passes obliquely through each ray, the main portion of the calcification lying external to it. On the assumption that the part marked *? p* is cartilage of perichondrial origin, one would conclude that the major portion of the calcified ray is also of perichondrial origin, and the deduction is strengthened by the discontinuity between the tesseræ of the basidorsal and basiventral cartilages, but the general appearances strongly suggest that the rays are calcifications in the arch-cartilages themselves (fig. 24, B, *? p'*, *? bd*).

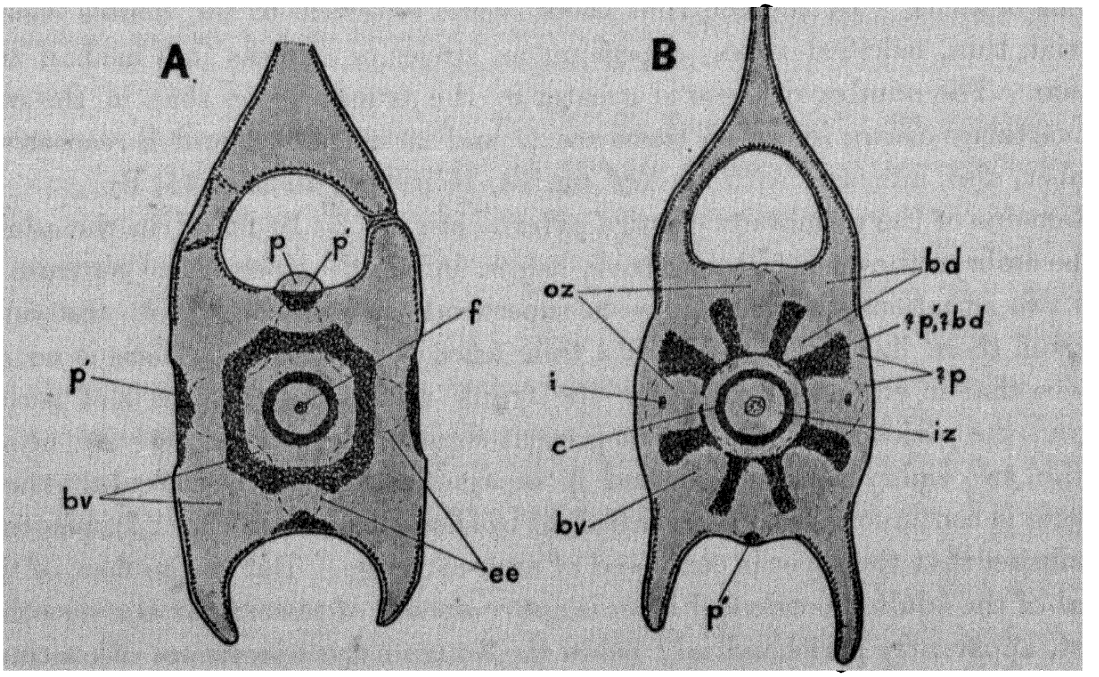


FIG. 24.—A, *Pristiophorus japonicus*, vertebra from region between pelvic and second dorsal fins, × 7. B, *Pliotrema warreni*, vertebra from region just behind first dorsal fin, × 7·5. (For explanation of the lettering, see p. 404.)

Returning to *Pristiophorus* (fig. 24, A), it will be noticed that the double projections from the main ring in the diagonal positions are the counterparts of the basal portions of the eight rays of *Pliotrema*, and there can be no question here as to

the projections being parts that belong to the arch-cartilages. The detailed study of the development of the vertebræ of both *Pristiophorus* and *Pliotrema* is greatly to be desired. The only young specimen available was one of *Pristiophorus cirratus*; although the fish was fairly large, measuring 300 mm. in total length, there is in the vertebræ no calcification, except that of the double cone.

The description given by HASWELL* is not clear, for, although he writes that the vertebræ of *Pristiophorus* are "devoid of radiating lamellæ," he says that in transverse section they "exhibit, as in the case of *Selache*, two pairs of cartilaginous tracts, passing from near the centre to the supero-lateral and infero-lateral parts of the vertebræ."

Squatinidæ.

The material available consisted of:—

Squatina squatina, vertebral column of adult, in alcohol, Jersey, presented by J. HORNELL, Esq.; vertebral column of adult, Oxford Univ. Museum, lent by Prof. E. S. GOODRICH; ripe embryo, 264 mm. long, purchased from Marine Biol. Lab., Plymouth; a younger embryo, Naples, presented by Prof. W. N. F. WOODLAND.

The vertebræ of *Squatina* are peculiar, and unique in character, and there are no connecting links that would serve to associate them with the vertebræ of other Elasmobranchs. In the centrum there occur, external to the double cone, several coaxial, thin, calcified tubes, appearing as circles or ellipses in a median transverse section. The number of these is greater in the trunk region than in the caudal; in the vertebræ drawn in fig. 25 there are 27 and 23 rings in A and B respectively; the number, also, increases with age (*cf.* fig. 13, B, p. 360, and fig. 25, B).

Remains of the membrana elastica externa are not to be found in the adult, but in a ripe embryo they can be seen above, below, and at the sides of the centrum. In the first two situations the membrane is superficial (fig. 13, B), but at the sides of the centrum there lies externally to it a thin tract of cartilage.† There is no reason to believe that in either the caudal or the trunk vertebræ this tract is of perichondrial origin; the perichondrium does not present signs of exceptional activity, at all events in the two embryos examined, and it is significant that in the adult the layer of tesseræ is continuous from basiventral to basidorsal cartilage (fig. 25), pointing to the conclusion that the layer is composed of arch-cartilage. But in the floor of the neural canal of the adult the calcified layer is not composed of tesseræ; it is a smooth calcified crust, apparently perichondrial; below the centrum the tesseræ are discontinuous, and there are no superficial calcifications near the median plane; in other words, there is no evidence of fusion between the two basidorsal cartilages, nor between the two basiventrals.

* 'Proc. Linn. Soc., N.S.W.,' ix, 1884, pp. 115 and 100.

† Similar relations are shown in the trunk vertebra of an embryo, 160 mm. long, figured by GOETTE (13, Plate 31, fig. 43).

SCHAUINSLAND (30, p. 413), following GOETTE (13), attributes the union of the basidorsal with the basiventral cartilage at the side of the centrum to the high position of the basiventral cartilage, resulting from the general flattening of the body, much the same as occurs in the Batoid fishes; but this does not explain the union of the two cartilages in the caudal region.

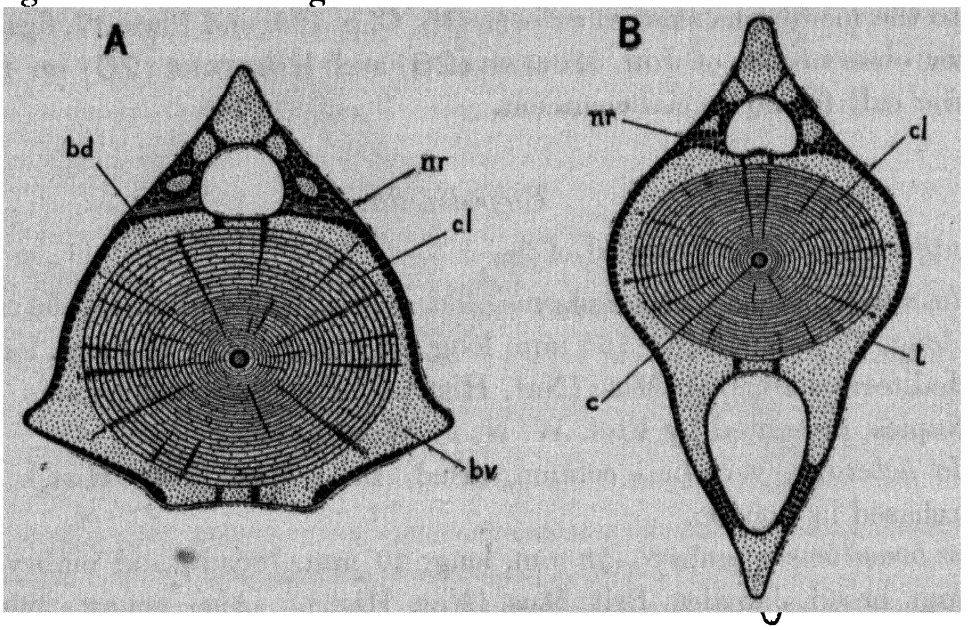


FIG. 25.—*Squatina squatina*, × 2. A, mid-trunk vertebra B, anterior caudal vertebra. (For explanation of the lettering, see p. 404.)

The caudal vertebra shown in fig. 13, B, exhibits four calcified rings external to the double cone.* Outside the outermost ring, and internal to the remains of the membrana elastica externa, in the position marked *g*, is a growing zone, in the inner part of which the fifth ring will be developed, while the outer part will persist as the growing zone. The presumption is that this growing zone, now in the outer-zone cartilage, remains morphologically within the membrana elastica externa, even after the remnants of that membrane are no longer to be detected; that is to say, since in fig. 13, B, the four rings belong to the sheath-cartilage, the presumption is that all the 23 rings shown in fig. 25, B, are of sheath-origin. If this be admitted as a valid argument, then *Squatina* is one of the most chorda-centrous of the Elasmobranch fishes.

Militating against the acceptance of this view, though perhaps not seriously, is the fact that, in the centra of the embryo, portions of the membrana elastica externa may occasionally be found entangled and left behind in the older, more central parts of the outer-zone cartilage; the growing zone, that is to say, may in some places spread to the outer side of the membrane.

In the adult the radiating markings that are seen in a transverse section are

* The trunk vertebræ of this fish have five rings, the vertebræ in the middle of the caudal fin only three.

partially calcified tubes containing blood-vessels that penetrate from without, somewhat similar to those seen passing through the concentric lamellæ of *Cetorhinus* (fig. 14, A, p. 361), although less numerous.

HASSE takes the view adopted above, that the calcified lamellæ of *Squatina* belong to outer-zone cartilage, and that there is a growing zone situated immediately internal to the membrana elastica externa (**15**, C, p. 129, and Plate 17, figs. 3 and 4, *a*). The earlier observations of JOH. MÜLLER (**26**) and KÖLLIKER (**20**) on the vertebræ of *Squatina* call for no special comment.

Torpedinidæ.

The material available consisted of:—

Torpedo narce, adult, also embryos and young, 82 mm. long, 50 mm. broad; 110 mm. long, 58 mm. broad; 165 mm. long, 93 mm. broad; 223 mm. long, 133 mm. broad; Mediterranean, Brit. Mus. (Nat. Hist.). Also vertebral column of adult, in alcohol, Naples, presented by Prof. W. N. F. WOODLAND.

Torpedo hebetans, vertebral column, dried, Brit. Mus. (Nat. Hist.). Also fresh adult, purchased in London.

Narcine brasiliensis, embryo, 53 mm. long, 20 mm. broad; and embryo, 104 mm. long, 58 mm. broad, Jamaica, Brit. Mus. (Nat. Hist.). Also young, 220 mm. long, 105 mm. broad, no locality, Brit. Mus. (Nat. Hist.).

Hypnos subnigrum, embryo, 65 mm. long, 42 mm. broad, Sydney, in the collection at the Royal Coll. Science.

The Batoid fishes, of which the Torpedinidæ are the first to be considered, require to be judged on their own merits, apart from the sharks, for, while their caudal vertebræ are largely comparable with those of sharks, the trunk vertebræ are greatly influenced by the extent to which the basiventral element has mounted up the side of the centrum and united with the basidorsal; this affects not only the mode of growth of the centrum, but particularly the pattern of the calcification. The interpretation of the vertebral structure of the Batoids is beset with difficulties, and the complete solution of the problem cannot be said to have been yet attained.

KLAATSCH has shown (**18**, II, pp. 168–170) that in *Torpedo* the immigration of cartilage-producing cells into the primary notochordal sheath occurs remarkably early (embryo, 26 mm. long), and the membrana elastica externa becomes reduced so soon that it cannot be relied upon as a distinctive delimiting layer. SCHAUINSLAND is of opinion (**30**, p. 418) that in the caudal as well as in the trunk region the basiventral is united with the basidorsal cartilage at the side of the centrum, and this at an early stage of development (embryo, 45 mm. long).

In an embryo of *Torpedo narce* measuring 82 mm. long and 50 mm. broad, the only parts of the membrana elastica externa to be recognised are those in the floor of

the neural canal and the roof of the hæmal canal. The secondary calcification is in the form of an investing layer, about twice as thick as the cone which it surrounds; the outline of its section is not strictly circular, but already shows indications of the six outgrowths which become more pronounced later.

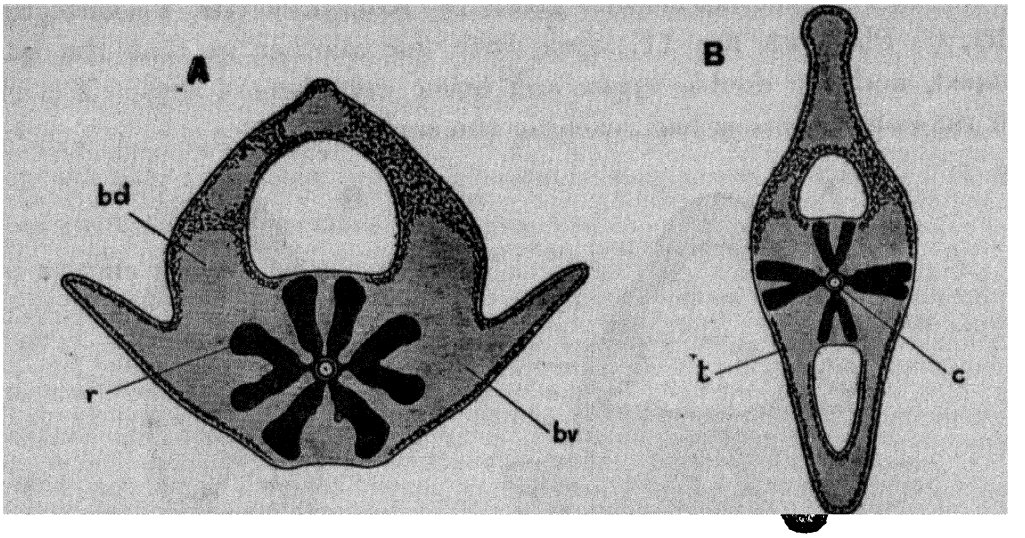


FIG. 26.—*Torpedo narce*, adult, × 7. A, mid-trunk vertebra. B, anterior caudal vertebra. (For explanation of the lettering, see p. 404.)

In the next stage available, an embryo measuring 110 mm. in length and 58 mm. in breadth, there are, in both trunk and caudal regions, six outgrowths of the secondary calcification, namely, two above, two below, and a massive one on each side (fig. 27, p. 380). [In GOETTE's figure of a young *Torpedo ocellata* (= *narce*), measuring 90 mm. in length, there are only four outgrowths, the upper and lower being single and broad (**13**, Plate 33, fig. 59), but he says (p. 489) that they branch later into a total of eight.] The parts of the membrana elastica externa seen in the earlier stage are still recognisable, and superficial in position (*e e*). In the two still later stages (165 × 93 mm., and 223 × 133 mm.) the six outgrowths are all radially longer, and they tend to enlarge at their outer ends, the lateral ones showing an indication of forking.

In the adult *Torpedo narce* (fig. 26) the lateral rays are well forked in the trunk vertebræ, less obviously so in the caudal. In the trunk vertebræ the outer ends of the rays are rounded and slightly enlarged. The upper and lower rays just fail to reach the surface; there are no tesseræ in these regions, and there are no evidences of perichondrial reinforcements such as occur in the three families that follow. In the caudal vertebræ also the rays fail to reach the surface; the tesseræ are discontinuous at the sides as well as above and below. The general impression conveyed by the sections, although there is no proof, is that the whole of the secondary calcification is of sheath-origin.

In the caudal vertebræ of *Torpedo hebetans* the transverse section exhibits four

short, stout calcified rays directed vertically and horizontally; their outer ends are swollen and are set at some little distance from the surface of the centrum. In the trunk region the lateral rays tend to fork and become Y-shaped, but the vertical rays show little indication of division; they merely broaden out.

The figures of *Torpedo marmorata* given by KOLLIKER (**19**, Plate 3, fig. 5) and HASSE (**15**, C, Plate 23, fig. 11) agree with one another in that the lateral rays are Y-shaped, and the double upper and lower rays form a letter X; the upper and lower rays are drawn as just reaching the surface.

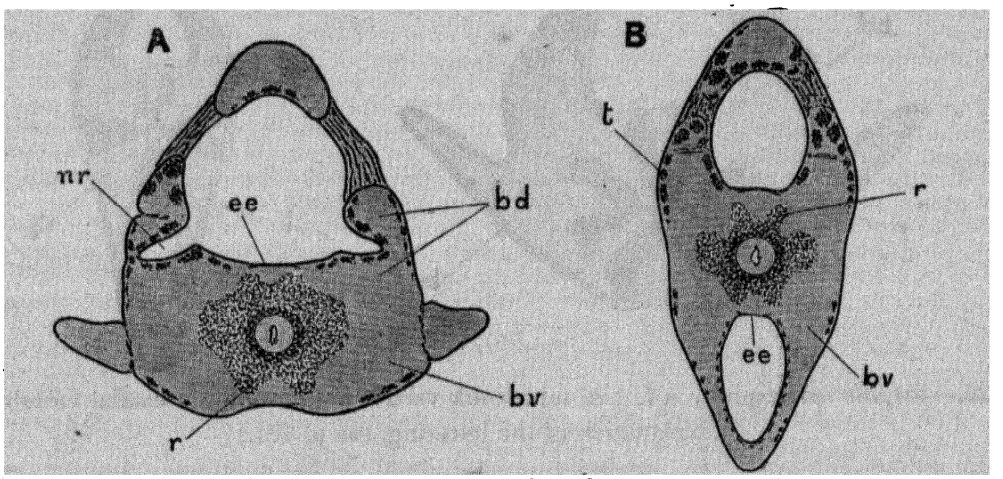


FIG. 27.—*Torpedo narce*, young, 110 mm. long, 58 mm. broad, × 18. A, mid-trunk vertebra. B, vertebra from region of first dorsal fin. (For explanation of the lettering, see p. 404.)

In *Narcine brasiliensis* there is some evidence, such as is not forthcoming in the case of *Torpedo*, that where the calcified secondary rays come near enough to the surface of the centrum they are reinforced by the perichondrium: this is shown in the third stage available (young, 220 mm. long, 105 mm. broad). In the first stage (53 × 20 mm.) the secondary calcification is merely an investing layer, with roughly circular outline; in the second stage (104 × 58 mm.) there are outgrowths somewhat similar to those shown in the figure of young *Torpedo* (fig. 27, B), except that they are less strongly developed.

If the fossil vertebra figured by HASSE (**15**, C, Plate 23, fig. 23) is correctly referred to *Narcine*, it shows that there is a very much greater complexity in this extinct species than in the modern forms.

Rhinobatidæ.

The material available consisted of—

Pristinæ.

Pristis cuspidatus, two, length from base of rostral saw to tip of tail about one metre, S. India, vertebral columns in alcohol, presented by J. HORNELL, Esq.;

also late embryo, total length 350 mm., of which the saw measures 115 mm., Travancore, Brit. Mus. (Nat. Hist.).

Pristis pectinatus, embryo, total length 265 mm., of which the saw measures 85 mm., no locality, Brit. Mus. (Nat. Hist.).

Pristis perrotteti, dried vertebræ, Brit. Mus. (Nat. Hist.).

Rhinobatinæ.

Rhynchobatus djeddensis, young, 685 mm. long, Tuticorin, S. India, vertebral column in alcohol, presented by J. HORNELL, Esq.; also embryo, 102 mm. long, Kurrachee, Brit. Mus. (Nat. Hist.).

Rhinobatus halavi, dried vertebræ, Brit. Mus. (Nat. Hist.).

Rhinobatus granulatus, skeleton in alcohol, Oxford Univ. Museum, lent by Prof. E. S. GOODRICH; also young, 233 mm. long, 82 mm. broad, in the collection at the Royal Coll. Science.

Rhinobatus undulatus, ripe embryo, 220 mm. long, Rio Grande do Sol, Brit. Mus. (Nat. Hist.).

Trygonorhina fasciata, young, 265 mm. long, 108 mm. broad, Sydney, in the collection at the Royal Coll. Science.

The caudal vertebræ of *Rhinobatus granulatus* (fig. 30, A, p. 383) or *Trygonorhina fasciata* (**15**, C, Plate 15, fig. 49) may be looked upon as the most generalised of the vertebræ of the Batoid fishes, and most characteristic of the sub-order. There are eight rays seen in the transverse section, four being diagonal, and not reaching the surface, while the other four, vertical and horizontal, reach the surface, and are reinforced by perichondrial increments. In a young *Rhinobatus*, measuring 233 mm. in length and 82 mm. in breadth, the secondary calcification, in the form of a gradually thickening investing layer around the double cone, has just reached the surface in four places (fig. 28, C, *p*), and in these places there is histological evidence to show that the perichondrium is beginning to be active. In earlier stages of development the growing zone is most probably circular in section, and situated immediately external to the calcified investing layer; but when this circular growing zone in the course of its enlargement approaches the surface of the centrum at the four parts indicated, the perichondrium immediately adjacent becomes rejuvenated, as it were, and in sections exhibits signs of activity, in marked contrast with the indifferent appearance of the general perichondrium that lies external to the parts where there are tesseræ.

In the trunk region of the same fish (fig. 28, B) much the same stage has been reached, but the lower tract of active perichondrium is very extensive, and the ventral tesseræ are more widely separated; and, owing to the union of the basi-ventral with the basidorsal cartilage, the growing zone does not reach the surface laterally. The lower edge of these sections is of particular interest, in that it shows

a duplicity of genetic tissue which cannot but be very transient. Immediately ventral to the calcified mass is a thin growing zone of small cells, below this is a thin

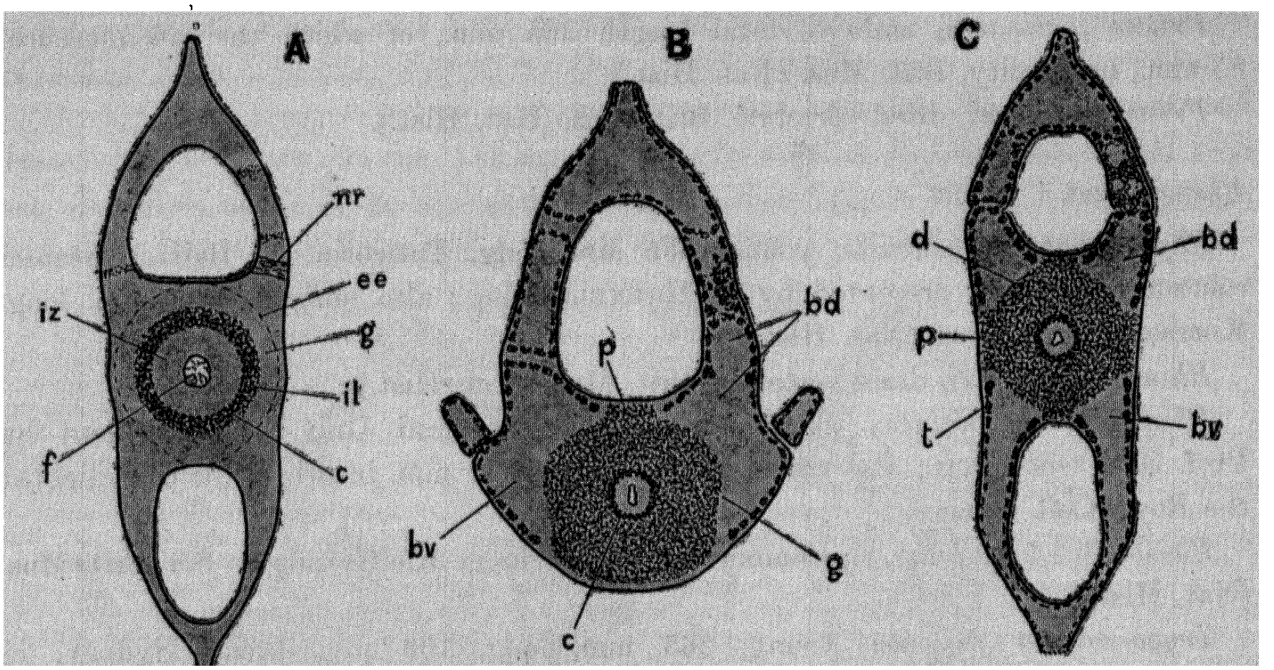


FIG. 28.—A, *Rhynchobatus djeddensis*, embryo, 102 mm. long; vertebra from region behind first dorsal fin, × 25. B, *Rhinobatus granulatus*, young, 233 mm. long, 82 mm. broad; mid-trunk vertebra, × 15. C, from region between pelvic and first dorsal fins, × 15. (For explanation of the lettering, see p. 404.)

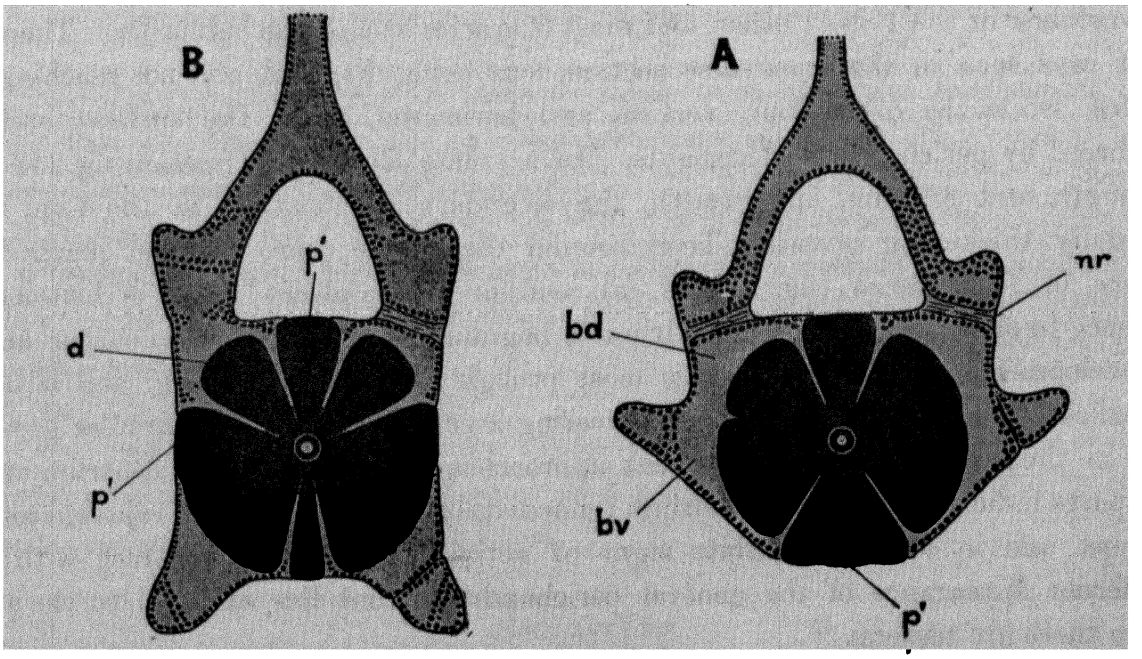


FIG. 29.—*Rhinobatus granulatus*, × 3. A, a mid-trunk vertebra, the second free vertebra behind the fused mass that is continuous with the back of the skull. B, vertebra from cloacal region. (For explanation of the lettering, see p. 404.)

tract of cartilage-matrix with few cells, and below this again is an active genetic layer, which a careful scrutiny of the right and left ends shows to be continuous with

the general perichondrium. The activity of the original growing zone is, as it were, infectious, and has been imparted to the connective tissue lying external to the cartilage.

What follows next can only be surmised, until slightly older stages are available for study, but seeing that the calcified rays in the areas in question maintain their connection with the surface in the adult (fig. 30, A; fig. 29, A), the most natural conclusion to be drawn is that the increments that are made subsequently to the stage of development shown in fig. 28 are due to perichondrial activity. The more external parts of the horizontal and vertical rays of fig. 30, A, may thus be regarded as intermedialia, comparable with those of *Mustelus* (fig. 8, A, *p'*, p. 354).

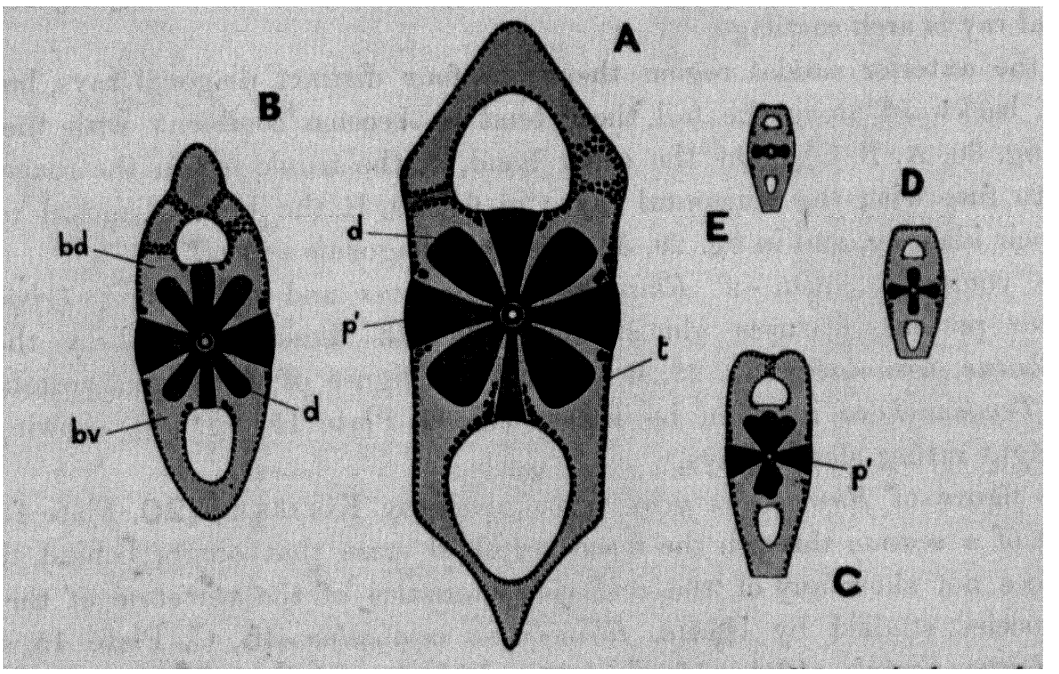


FIG. 30. *Rhinobatus granulatus*, × 3. A, caudal vertebra, taken a short distance in front of the first dorsal fin. B, vertebra from the base of the caudal fin. C, from half way between the base of the caudal fin and the extreme hind end of the vertebral column. D, from about half-way between C and the extreme hind end of the vertebral column. E, vertebra a short distance behind D. (For explanation of the lettering, see p. 404.)

In support of this view is the fact that when, as in the vertical rays of fig. 30, D, the rays fail to reach the surface, their ends are rounded, and the perichondrium in the floor of the neural canal and in the roof of the hæmal canal does not differ histologically from the general external perichondrium; these rays, that is to say, grow by virtue of the original growing zone. In further support of the view is the fact that in some Batoids there is evidence that the perichondrial tracts may become active without experiencing the stimulus due to the approaching primary growing zone. In some of the caudal vertebræ of *Raia*, for instance, the inner and outer parts of the horizontal rays are discontinuous (fig. 32, D, E, p. 387); the

inner part has grown outward by increments from the primary growing zone, the superficial part is growing outward by virtue of a perichondrial meristem.

As regards the diagonal rays (fig. 30, A, *d*), there can be no doubt that they continue to grow from the original growing zone. The early disappearance of the membrana elastica externa renders it impossible to say whether there is, as in the Carchariidæ, a definite outward growth of sheath-cartilage, with the diagonal rays calcifying within it (fig. 8), or whether there is a spreading of the ray into the arch-cartilage; all that is certain is that the investing layer, from which they primarily arose, is a structure belonging to the outer-zone cartilage. The presence of tesseræ in fig. 30, A, in the interval between the lower vertical and lower diagonal rays rather suggests that the part of the cartilage that is immediately adjacent to the vertical ray is arch-cartilage.

In the anterior caudal region there are four distinct diagonal rays, but as one passes backward along the tail these tend to become confluent with the vertical rays (fig. 30, A, B, C). On the other hand, in the trunk region the diagonal rays tend to fuse with the horizontal rays; in fig. 29, B, the lower diagonal rays have lost their identity, and in fig. 29, A, the upper diagonals as well.

The young specimen of *Rhinobatus undulatus* and the young *Trygonorhina fasciata* present features almost identical with those exhibited by the young *Rhinobatus granulatus* (fig. 28, B and C). A figure of the caudal vertebra of an adult *Trygonorhina* is given by Hasse (**15**, C, Plate 15, fig. 49), showing a star with eight rather slender rays.

The figure of *Rhinobatus granulatus* given by Kölliker (**20**, Plate 13, fig. 2), is that of a section through the fused vertebral mass that occurs behind the skull, and does not show any of the real characteristics of the vertebræ of the species. The species studied by Hasse, *Rhinobatus cemiculus* (**15**, C, Plate 15, fig. 41), *Rhinobatus horkeli* (Plate 15, fig. 27), *Rhinobatus thouini* (Plate 14, fig. 14), constitute a graded series, the first of which has an open eight-rayed star, like *Trygonorhina*, and the last a compact calcareous mass, like *Pristis*. The differences observable within the limits of the genus are very remarkable.

The vertebræ of adult specimens of *Pristis cuspidatus* (fig. 31) and *Rhynchobatus djeddensis* are similar in possessing a compact calcified mass, with a circular outline as seen in transverse section; in those cases in which the circular outline is not strictly adhered to there is a tendency for the mass to bulge out in the horizontal and vertical lines, as if to form horizontal and vertical rays. The layer of tesseræ is usually, though not invariably, incomplete in these parts, but in the vertebræ of the trunk region the lateral layers of tesseræ are almost always continuous. In the hindermost caudal vertebræ the pattern gives place to a simple cross of vertical and horizontal rays, which do not reach the surface (fig. 31, E); this occurs in *Rhynchobatus* as well as in *Pristis*.

The relation of the compact mass of *Pristis* to the more typical eight-rayed cross

is explained by reference to *Rhinobatus*. In the trunk vertebræ of *Rhinobatus granulatus* there is a tendency for the rays to broaden out and coalesce, and, as is shown by fig. 29, A, where the horizontal and diagonal rays are amalgamated, it only needs the further coalescence of the lateral calcified masses with the vertical rays to produce the effect found in *Pristis*. The series of the three species of *Rhinobatus* studied by HASSE (*supra*) is another illustration of the same point.

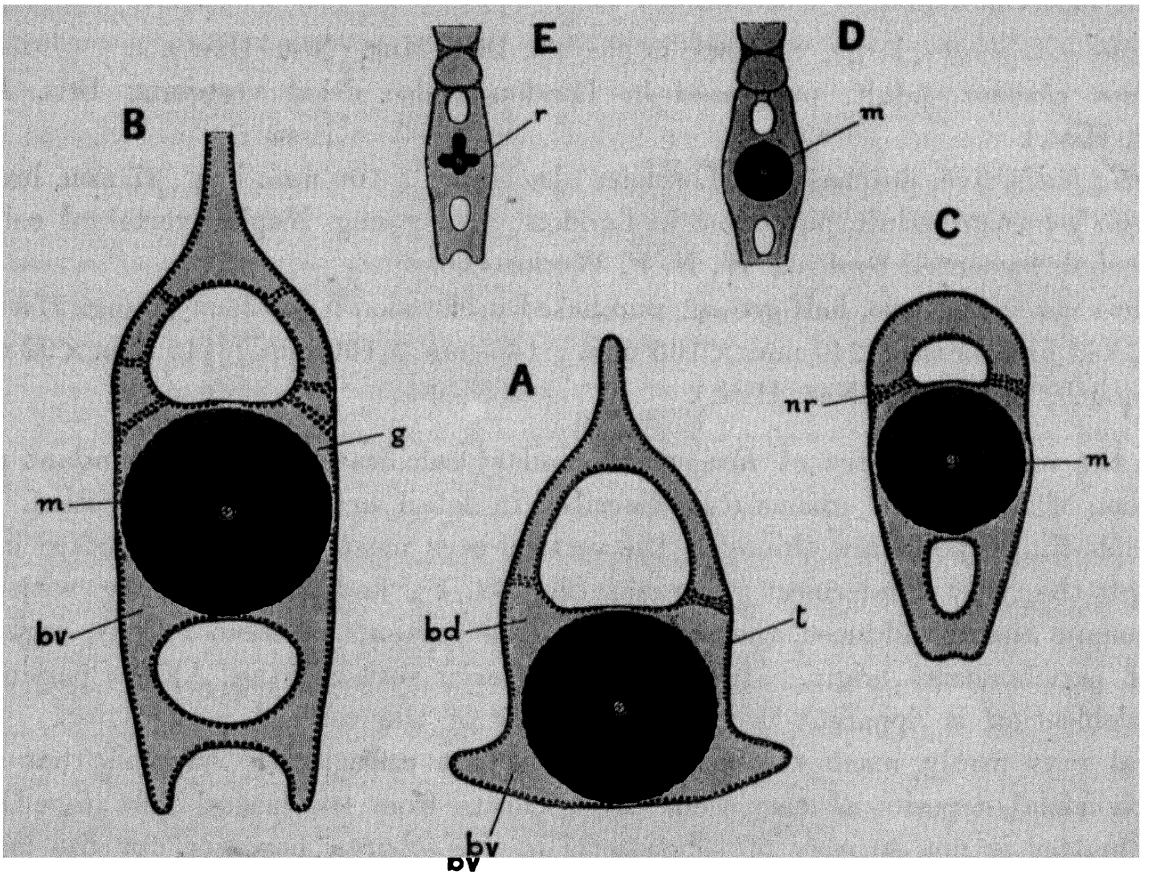


FIG. 31.—*Pristis cuspidatus*, half-grown fish, measuring about 1 metre from base of rostral saw to tip of tail. A, mid-trunk vertebra, × 3. B, vertebra from cloacal region, × 3. C, vertebra from root of caudal fin, × 3. D, vertebra from middle of caudal fin, × 4·5. E, vertebra near the posterior extremity of the vertebral column, × 4·5. (For explanation of the lettering, see p. 404.)

In the embryonic specimen of *Rhynchobatus djeddensis* (fig. 28, A), the membrana elastica externa is still visible; it is superficial above and below, but at the sides there is a thin tract of cartilage lying external to it. The double cone has only a thin investing layer, scarcely yet calcified, and between the latter and the membrana elastica externa is the zone of growth. Seeing that the growing zone is at present within the outer-zone cartilage, and that its activity persists continuously throughout life, there would appear to be some justification in regarding those later deposits of the calcified mass of the adult that are produced after the membrana elastica externa has disappeared as of sheath origin also.

The embryo of *Pristis pectinatus* examined exhibits a later stage, the investing

layer being comparatively thick and well calcified; the membrana elastica externa is still visible, and lies external to the growing zone. In the late embryo of *Pristis cuspidatus* studied there are no signs of the membrana elastica externa, and the calcified mass has already acquired a close resemblance to that found in the adult.

Raiidæ.

The material available consisted of:—

Raia marginata, large, vertebræ in alcohol, Brit. Mus. (Nat. Hist.).

Raia clavata, adult, purchased in London; also dried vertebræ, Brit. Mus. (Nat. Hist.).

Raia batis, five, purchased in London; also embryo, 100 mm. long, 37 mm. broad.

Raia punctata, adult, purchased in London; also young, Naples, vertebral column in alcohol, presented by Prof. W. N. F. Woodland.

Raia maculata, two, half-grown, purchased in London; also four young, 275 mm. long, 185 mm. broad; 240 mm. × 165 mm.; 145 mm. × 100 mm.; 145 mm. × 92 mm., Plymouth, Brit. Mus. (Nat. Hist.).

In the vertebral centra of *Raia* the secondary calcification is less abundant than in those of *Rhinobatus granulatus*, described in detail in the previous section. All the radiating lamellæ are thinner; the vertical rays mostly reach the surface in all except the very hindermost vertebræ (fig. 32, F), and in conformity with the arguments already adduced, the more superficial portions of them may be regarded as of perichondrial origin. In the most anterior vertebræ (fig. A) the parsimony of calcification is apparent from the tendency of the vertical rays to fork. The lateral rays rarely reach the surface, and it not infrequently happens that such perichondrial deposits as may occur are separate from the lateral rays (figs. D, E). The matter is not so easy of solution as in *Rhinobatus*, however, for the lateral tesseræ are early continuous (fig. 34), and one must admit, in consequence, that there is some arch-component in the lateral calcified masses.

Diagonal rays as such cannot be distinguished; one must rather say that there are lateral parts which fork and branch irregularly. The irregularity is here illustrated not only by fig. 32, A–D, but by fig. 33. The upper row in fig. 33 represents the calcified star in four selected vertebræ of a large specimen of *Raia marginata*; and the series may be amplified by the addition of fig. 4, B, p. 340, which is drawn from an anterior trunk vertebra of the same fish. This last figure differs from the first of fig. 33 in having the upper vertical ray branched.

The lower four horizontal rows of fig. 33 illustrate the considerable variation that may be observed in different individuals of the same species; the figures are strictly comparable, since the vertebræ were selected from corresponding regions of the body in the four fishes. The specimen of *Raia punctata* examined had vertebræ agreeing with those shown in the third horizontal row.

In vertebræ of *Raia batis* from the region just behind the second dorsal fin there is no internal calcification except the double cone. Within the double cone is a long plug of inner-zone cartilage, which terminates abruptly in front and behind; the remains of the notochord in the intervertebral regions and in the broadest parts of the cones is watery rather than gelatinous. In vertebræ still more posterior the narrowest part of the double cone is granular and irregular, and in dried preparations from which the cartilage has been removed the two cones break apart. The last ten or twelve vertebræ of the tail have no calcification in the interior of the centrum.

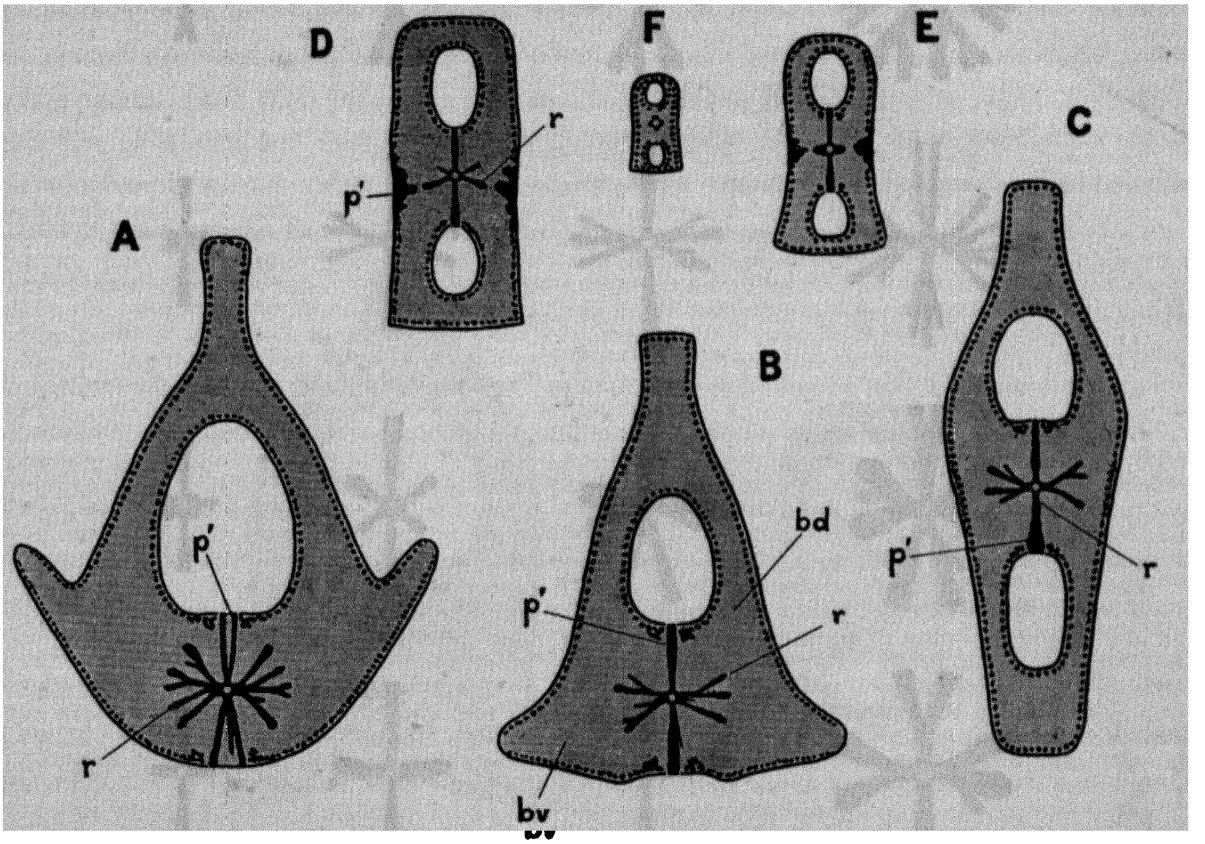


FIG. 32.—*Raia batis*, × 1·5. A, mid-trunk vertebra. B, vertebra from cloacal region. C, post cloacal vertebra. D, vertebra from region midway between cloaca and first dorsal fin. E, vertebra from region of first dorsal fin. F, vertebra from region of second dorsal fin. (For explanation of the lettering, see p. 404.)

In the absence of any evidence to the contrary, it may be taken that the lateral rays that do not reach the surface—they do so occasionally in the caudal vertebræ—are structures belonging to the outer zone of the sheath; this is the view taken by HASSE (**15**, C, Plate 22, fig. 11, *a'*).

In a young *Raia maculata*, measuring 145 mm. in length and 100 mm. in breadth, the membrana elastica externa is still recognisable. Vertebræ from the region of the second dorsal fin show the membrane superficial in the floor of the neural canal and in the roof of the haemal canal; there are no tesseræ in these positions. At the sides of the centrum the tesseræ are continuous, and a thin tract of cartilage

occurs between them and the membrana elastica externa. There is thus evidence here, as already pointed out by previous writers, of a union of the basidorsal and basiventral cartilages; there is no evidence, however, of confluence of the right and left basidorsal cartilages, nor of the two basiventral cartilages. The double cone is surrounded by a very thin investing layer.

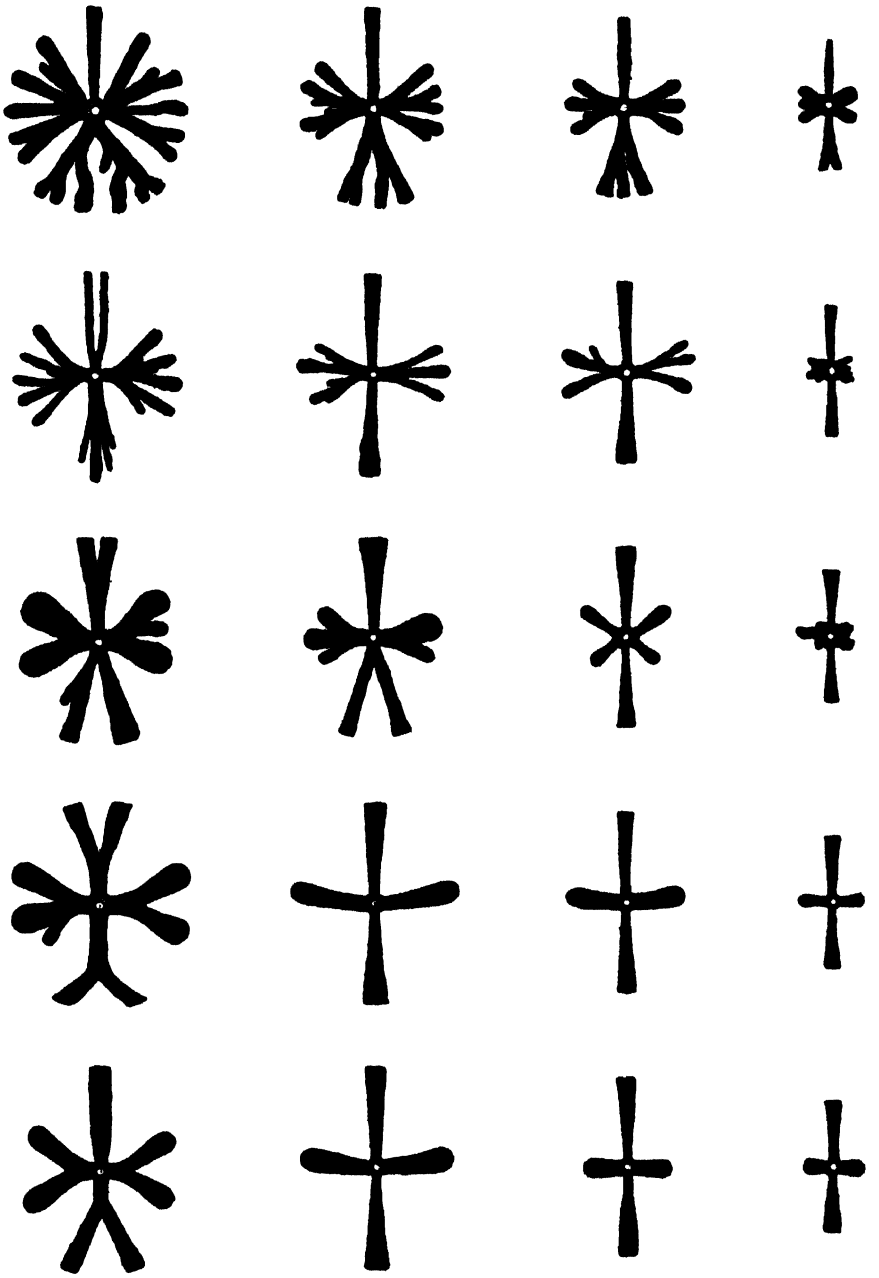


FIG. 33.—Patterns of the calcified rays of the centra of *Raia*, as seen in transverse sections. Upper horizontal row, *Raia marginata*; other rows, *Raia batis*. Left-hand vertical row, mid-trunk vertebræ; second row, vertebræ from cloacal region; third row, post-cloacal vertebræ; right-hand row, vertebræ from region midway between cloaca and first dorsal fin.

A vertebra from the region of the first dorsal fin (fig. 34, B) shows, between the lateral tesseræ and the membrana elastica externa, a wider tract of cartilage than

in the vertebra mentioned above. The investing layer is not circular in outline, but exhibits signs of the early development of the upper and lower vertical rays, the lower rudiment being in some cases discontinuous from the general investing layer. Since the upper and lower parts of the membrana elastica externa are still visible and superficial, it is clear that these basal parts of the future vertical rays are derived from sheath-cartilage, and the remarks as to perichondrial increments apply only to the later developed, more distal portions.

A vertebra from the cloacal region (fig. 34, A) shows a thicker investing layer than the last, and there are no longer any signs of the membrana elastica externa in the dorsal and ventral positions; in each of these positions there is a small-celled growing zone, but the evidence is less clear than in *Rhinobatus* that the perichondrium is about to reinforce the original genetic tissue. This particular stage of development is not quite advanced enough to enable one to prove that similar conditions will prevail in *Raia*.

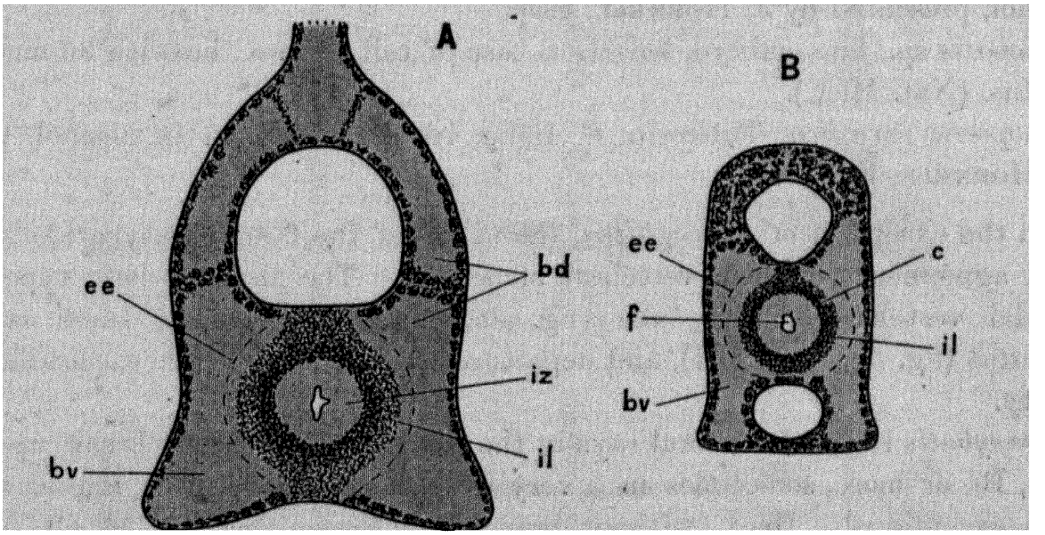


FIG. 34.—*Raia maculata*, young, 145 mm. long, 100 mm. broad, × 24. A, vertebra from cloacal region. B, from region of first dorsal fin. (For explanation of the lettering, see p. 404.)

In his description of the vertebral structure of a young *Raia clavata*, 120 mm. in length, GOETTE (**13**, p. 489, and Plate 32, fig. 54) refers to an independent origin of the rays; he shows four small calcified patches in diagonal positions, and isolated from the double cone. But it would seem that the section is taken, not through a trunk vertebra, but through the fused vertebral mass that occurs behind the skull. The caudal vertebra of an embryo, measuring 70 mm., also figured by GOETTE (Plate 32, fig. 47), does not differ materially from the vertebra described above from the region of the second dorsal fin of *Raia maculata*.

Dasybatidæ.

The material available consisted of:—

Pteroplatea micrura, two, vertebral columns in alcohol, Madras, presented by J. Hornell, Esq.

Urolophus (*Trygonoptera*) *testacea*, late embryo, length to base of tail 75 mm., breadth 50 mm., Sydney, presented by Prof. J. P. Hill.

Dasybatis sp., embryo, length to base of tail 45 mm., breadth 50 mm., no locality, Brit. Mus. (Nat. Hist.).

Dasybatis thalassia, two, dried vertebræ, Brit. Mus. (Nat. Hist.).

Urogymnus asperrimus, dried vertebræ, Brit. Mus. (Nat. Hist.).

Myliobatis aquila, young, length to base of tail 185 mm., in the collection at the Royal Coll. Science.

Myliobatis nieuhofi, young, breadth 330 mm., Tuticorin, S. India, vertebral column in alcohol, presented by J. Hornell, Esq.

Myliobatis sp., late embryo, length to base of tail 70 mm., breadth 90 mm., Japan, Brit. Mus. (Nat. Hist.).

Rhinoptera javanica, Tuticorin, S. India, vertebral column in alcohol, presented by J. Hornell, Esq.

With the exception of *Pteroplatea*, the fishes of the family Dasybatidæ exhibit a general agreement in their vertebral structure. The characteristic type, as seen in caudal vertebræ of *Dasybatis* (fig. 35, A), is clearly the same as that of *Rhinobatus* (fig. 30, A, p. 383), and departures from this type are explicable without difficulty.

In *Dasybatis* itself the lateral rays in the caudal vertebræ may break up into two (fig. 35, B), or more, sometimes in a very irregular manner. The lateral rays may become exceptionally thick, without branching (*Rhinoptera*, fig. 36, C), or it may happen that the diagonal rays thicken, while the horizontal and vertical rays remain thin (*Urogymnus*, fig. 36, A).

There is a tendency, particularly in *Dasybatis* (fig. 35), for the outer parts of the vertical and horizontal rays to be flanked by tesseræ, indicating that those parts are situated *between* arch-components, which they prevent from coalescing. Such outer parts may be regarded as of perichondrial origin, although the more central parts of the same rays are clearly calcifications within sheath-cartilage. When, as in the caudal vertebræ of *Myliobatis* (fig. 37, B), the vertical and lateral rays do not reach the surface, a doubt is introduced, for while one might argue that the outer parts of the rays are here of sheath-origin, the possibility is not excluded (*infra*) that the tract of cartilage lying beyond the peripheral end of each may be uncalcified cartilage of perichondrial origin, and that the outer part of the calcified ray may have originated from the same genetic tissue.

In the specimen of *Myliobatis* examined by Hasse (**15**, C, p. 151, and Plate 20,

fig. 3), the vertical rays are less distinct, and the outline of the calcified mass is more circular, rather resembling that of *Pristis* (fig. 31, C, p. 385). KÖLLIKER, on

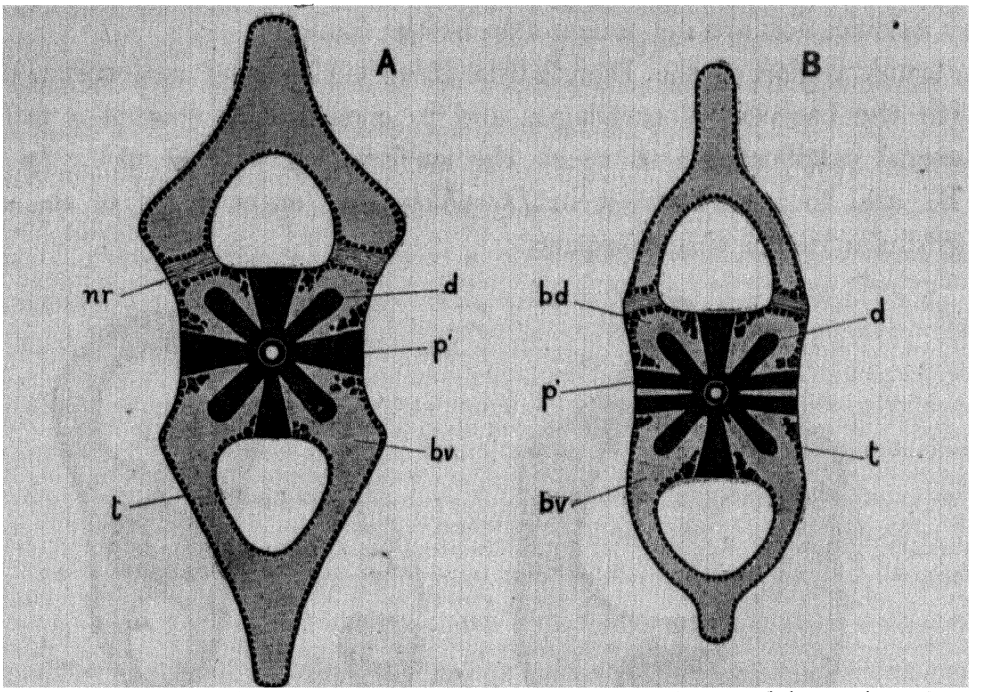


FIG. 35.—*Dasybatis thalassia.* Composite figures of several consecutive caudal vertebræ, from the region just in front of the tail-spines. A, from a large specimen, × 2. B, from a smaller fish, × 2. (For explanation of the lettering, see p. 404.)

the other hand, writes that the young *Myliobatis* that he studied agrees with *Rhinobatus granulatus* in its vertebral structure (**20**, p. 55).

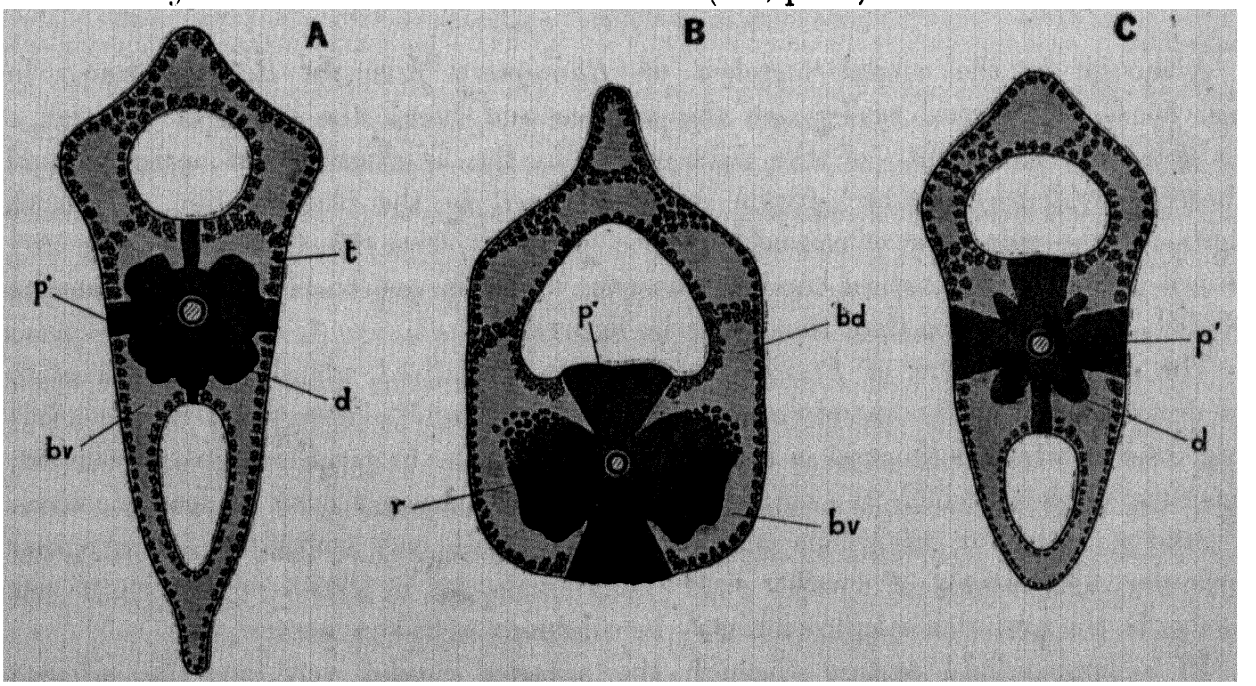


FIG. 36.—A, *Urogymnus asperrimus*, vertebra from base of tail, × 4·2. B, *Rhinoptera javanica*, pre-cloacal vertebra, × 4·2. C, from region immediately behind the dorsal fin, × 4·2. (For explanation of the lettering, see p. 404.)

Judging from HASSE's figures of caudal vertebræ of *Hypolophus*, *Urolophus* and *Taeniura* (**15**, C, Plate 19, figs. 3, 9, 16), it would seem that instances of excessive secondary calcification are not uncommon in the family.

In the trunk region of the Dasybatidæ there is the usual coalescence of the basidorsal with the basiventral cartilages, and in consequence thereof a failure of any of the lateral calcifications to reach the surface (*e.g.*, fig. 37, A). In *Rhinoptera* (fig. 36, B), and to a less extent in *Dasybatis*, the outer parts of the calcification may be granular rather than compact.

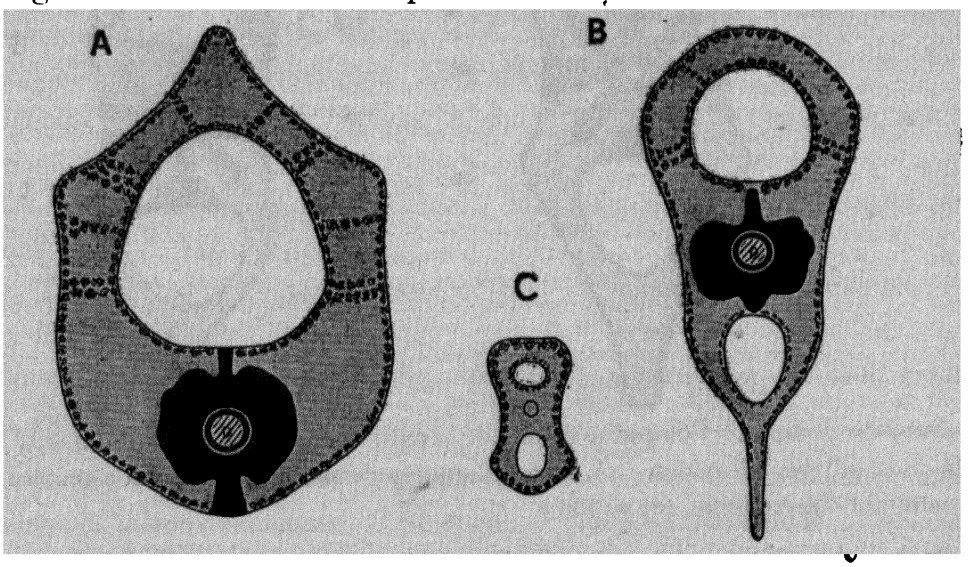


FIG. 37. —*Myliobatis meuhofi*, 330 mm. broad, × 9. A, pre-cloacal vertebra. B, post-cloacal vertebra, from region of dorsal fin. C, from basal part of the tail-whip.

Although in the caudal vertebra of *Rhinoptera javanica* that is shown in fig. 36, C, the lateral rays reach the surface and break the series of tesseræ, a reduction in the length of the rays occurs in the anterior caudal vertebræ, and there is evidence that arch-fusion is not confined to the trunk region. HASSE's figure of the side view of caudal vertebræ of *Rhinoptera* (**15**, C, Plate 21, fig. 26), shows a continuous vertical band of tesseræ, which is not to be found in vertebræ slightly more posterior than those that he figures.

The vertebral centra of *Pteroplatea* are very specialised, mainly in the direction of degeneration. The membrana elastica externa can be recognised in the adult (fig. 38, *ee*), which is unusual in Batoids ; and within it are large, irregularly shaped, granular deposits, which in a transverse section are disposed more or less in a circle around a central cartilaginous core. The core itself may contain a few irregular granular calcifications of smaller size. Occasionally, as is shown on the right side of fig. B, the granular calcification may be confluent with the tesseræ.

In a longitudinal section through the anterior caudal vertebræ the calcified nodules are seen to be set lengthwise in series of four, and the terminal members of the series are confluent with transverse calcified plates, which are evidently the

widely separated and greatly flattened cones of the centrum. The posterior plate of one centrum and the anterior plate of the next enclose between them some relics of the original notochordal substance. The granular nodules between the two flattened cones of the same centrum are comparable with the granular deposits found in the long terminal caudal vertebræ of *Raia* (p. 318).

Two specimens of *Pteroplatea micrura* were studied and the vertebral structure of each was as described above. HASSE, however, in a transverse section of a trunk vertebra (**15**, C, Plate 19, fig. 11), figures a continuous calcareous deposit around a thin double cone. The former he regards as an outer zone derivative (**15**, C, p. 141), which may be correct, but there is nothing in my own sections to prove it.

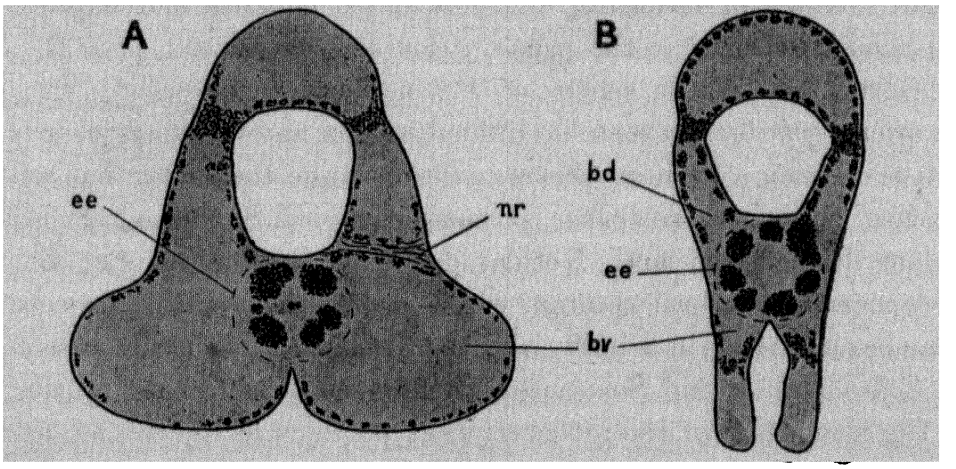


FIG. 38 *Pteroplatea micrura*, × 12. A, pre-cloacal vertebra. B, vertebra from region of tail spine (For explanation of the lettering, see p. 404.)

Of the embryos studied, that of *Urolophus* (*Trygonoptera*) *testacea*, 50 mm. in breadth, proves to be too young to show any calcification. The *Dasybatis* embryo, measuring 50 mm. in breadth and 45 mm. from snout to base of tail shows, in both trunk and caudal vertebræ, a well-formed double cone, but no investing layer; the membrana elastica externa is to be observed above and below the centrum, and occupies a superficial position, but the other parts of the membrane are no longer to be detected.

The embryo of *Myliobatis*, 90 mm. broad, shows a developing investing layer, which in the trunk region nearly extends to the surface above and below; the membrana elastic externa is recognisable above, and is superficial. In the caudal vertebræ at the base of the tail-whip the membrane is superficial above and below the centrum; it can also be recognised at the sides, and is there seen to be separated from the actual surface by about two layers of cartilage-cells, which create the impression that they have originated from the perichondrium, and not by a "creeping" of the basidorsal and basiventral cartilages towards one another. There is also evidence in these sections that, until the arch-cartilages have reached the

stage of development at which the tesseræ begin to calcify, parts of them are increased by perichondrial additions.

Conclusions as to the Taxonomic Value of the Calcified Parts of the Centra.

As regards the value that may be attached to the patterns of the calcified structures in the centrum as an aid to taxonomy, it may be pointed out, as one of the results of the present investigation, that species and genera, which on other grounds are considered to be nearly related, conform with one another fairly well in the matter of the calcification of the vertebral centra. The Squalines, for instance, agree in the absence of radiating and concentric lamellæ, and exhibit at most an "investing layer" external to the primary double cone (fig. 22, p. 372). The absence of calcified structures in the centra of *Echinorhinus spinosus* (fig. 23, p. 373), and *Somniosus microcephalus* presents no difficulties, for the former is clearly a degraded representative of such a form as *Centroscyllium*, while the latter has within its own genus a species, *Somniosus rostratus*, possessing typical Squaline vertebræ.

The Chlamydoselachidæ and Notidanidæ may in like manner be regarded as having a degenerate vertebral column, which in its more perfect form would exhibit calcified lamellæ radiating in a bulky sheath-cartilage. The evidences of degradation are, as in *Echinorhinus* and *Somniosus*, the partial liquefaction of the notochordal substance, the elongation of the intervertebral portions of the column at the expense of the vertebral, and the tendency to the development of large plate-like transverse septa in the vertebral regions. Further evidence is forthcoming in the occurrence, in the hinder caudal vertebræ of *Heptanchus*, of calcified double cones with radiating secondary lamellæ, usually six in number (fig. 5, A, p. 348). These calcified parts disappear as one passes forwards, and the observations should be taken in conjunction with the general conclusion, arrived at from other sources, that caudal vertebræ in sharks exhibit a more primitive or more typical condition of structure than the trunk vertebræ (p. 317).

The only other forms that exhibit radiating calcified lamellæ that belong exclusively to the sheath-cartilage are the various species of *Cestracion* (fig. 21, p. 370).* This is not, however, to be taken as necessarily implying close affinity with *Heptanchus*; indeed, the fact that the characteristic number of rays in *Heptanchus* is six, while in *Cestracion* it is eight, tells rather against it. The fossil genera, such as *Palæospinax*, that are commonly regarded as allied to *Cestracion*, although their centra have no radiating calcified laminæ, may possibly be degenerate, but the more probable explanation is that they branched off from the parental stock prior to the evolution in the Cestracionts proper of the calcified lamellæ external to the double cone.

* And possibly *Orectolobus* (see p. 396).

The members of the family Carchariidæ agree with one another in the possession of large, well calcified intermedialia, of perichondrial origin, not derived from the arch-cartilage nor from the sheath-cartilage; they also agree in the fact that the sheath-cartilage bulges out towards and into the arch-bases, and includes a diagonally placed calcified lamella in each outgrowth.

The Lamnidæ and Odontaspididæ agree among themselves, and differ from the Carchariidæ in the great reduction, or absence, of diagonal outgrowths of sheath-cartilage and their included calcified lamellæ, and they differ further from the Carchariidæ in the intermedialia not being compactly calcified, but consisting largely of cartilage. This cartilage of the intermediale is calcified on the faces that touch the arch-cartilages, and usually contains within it several radiating lamellæ (*Carcharodon*, fig. 9, A, p. 356), although these last are wanting in *Scapanorhynchus* (fig. 11, p. 358). *Cetorhinus* is exceptional in the lateness of the development of the radiating lamellæ of the intermedialia, all the early lamellæ being of the concentric type (fig. 13, A, p. 360). Even in the adult *Cetorhinus* (fig. 14, A, p. 361), and in some other forms, there is a tendency for the calcified parts of the intermedialia to exhibit a concentric arrangement. While in the Carchariidæ the calcified innermost parts of the intermedial wedges are separate from the investing layer, they are continuous with it in the Odontaspididæ and Lamnidæ (except young *Cetorhinus*).

These two families, the Lamnidæ and Odontaspididæ, are probably those in which, of all Elasmobranchii, the vertebræ are the least chordacentrous; the part of the centrum of the adult fish that is constructed out of the cartilage of the notochordal sheath is very small. There is nothing exceptional in the double cone and the inner-zone cartilage, but the outer-zone cartilage and its products are at a minimum of development.

The concentric lamellæ of *Squatina* are unique among Elasmobranchii, and the family Squatinidæ, so far as its vertebral structure is concerned, stands alone. The lamellæ are of sheath-origin, and are not to be confused with the early laminæ of *Cetorhinus*, which are developed in the intermedialia (fig. 13, p. 360).

As regards the Scyliorhinidæ, the typical vertebræ of the family are clearly those of such a species as *Scyliorhinus marmoratus* (fig. 15, p. 363), in which there is a very obvious approach to the Carchariidæ in the outward growth of the sheath-cartilage in the four diagonal positions, and the presence of a radiating calcified lamella in each outgrowth. The intermedialia are relatively large, and have the form of cartilaginous wedges, calcified on each surface that abuts upon arch-cartilage. The vertebræ of the British species, *Scyliorhinus canicula* and *Scyliorhinus stellaris*, depart from the type seen in *Scyliorhinus marmoratus* in the absence of the diagonal calcified lamellæ, and in the flattening out of the intermedialia, so that they extend only a short distance inward (fig. 16, A and B, p. 363); they are completely calcified, and at their edges they merge insensibly into the tesseræ of the arch-cartilages. In *Pseudotriacis* and *Pristiurus* the intermedialia are still further reduced, and the

condition of the vertebral centrum comes to resemble that found in the Squalinæ (*cf.* fig. 16, C, p. 363, and fig. 22, B, p. 372), a coincidence which is of no taxonomic importance.

In the family Orectolobidæ, the genera *Chiloscyllium*, *Stegostoma*, and *Ginglymostoma* agree in having radiating calcified lamellæ of perichondrial origin, usually commencing in eight regions (fig. 18, A and C, p. 366, and fig. 17, E and F, p. 365), but liable to fork or branch subsequently (fig. 17, A and B; and HASSE, **15**, D, Plate 26, fig. 24). They may be connected with the double cone by an investing layer (fig. 18), or may be independent of the double cone (fig. 17). The cartilage of the outer zone of the notochordal sheath bulges out horizontally and vertically into the intermedialia.

In *Orectolobus* a different type prevails, at all events in young and half-grown specimens. There are primarily eight radiating lamellæ (fig. 20, C, p. 368), but, so far as is shown by the position of the remnants of the membrana elastica externa that are still recognisable, these rays would seem to be developed within the outer-zone cartilage, and not to be of perichondrial origin. Whether in full-grown specimens outward growth of the rays is continued by perichondrial activity, is at present doubtful; HASSE'S figures (**15**, D, Plate 25, figs. 4–6) are drawn from vertebræ no larger than those of the 700 mm. specimen examined in the course of the present investigation, yet he represents the rays as reaching the surface, exactly as in *Stegostoma* and *Ginglymostoma* (*ibid.*, Plate 25, fig. 3, and Plate 26, fig. 21).

The vertebræ of *Rhinodon* do not seem to have been described; none were available for study in the present inquiry.

As regards family relationships, it is to be noted that while of the five families grouped by REGAN (**29**) in the Division Galeoidei the families Odontaspididæ, Lamnidæ, Scyliorhinidæ and Carchariidæ agree in the essential structural features of the vertebral centra, the family Orectolobidæ stands aloof.

There is nothing in the vertebral structure of the Pristiophorinæ to suggest that they are at all closely related to the Squaline sharks. Accepting the vertebral structure of *Pliotrema* as being more typical than that of *Pristiophorus* (*cf.* B and A, fig. 24, p. 375), there is to be noticed a resemblance to the Orectolobidæ in the manner in which the outer-zone cartilage bulges out horizontally and vertically. The eight calcified lamellæ bear some resemblance to those of a young *Chiloscyllium* (fig. 17, F, p. 365).

In the case of the Batoid fishes the characters of the vertebral centra do not appear to have any very direct bearing on the classification of the families within the limits of the sub-order. As a characteristic pattern for the Batoids one might select the eight-rayed star such as occurs in the anterior caudal vertebræ of *Rhinobatus* (fig. 30, A, p. 383). Of the eight rays, four are diagonal, and the others vertical and horizontal. The development of all of them commences in the outer-zone cartilage (fig. 28, C, p. 382), and the diagonal rays may continue to increase in the diagonally

expanding sheath-cartilage, much the same as in Carchariidæ, or they may possibly extend into the arch-cartilage; the early disappearance of the membrana elastica externa precludes one from arriving at any definite conclusion on the point. The horizontal and vertical rays continue to grow by perichondrial increments, and the outer parts of these rays thus resemble the horizontal and vertical rays of *Mustelus* (fig. 8, A, p. 354).

The practice of founding a scheme of classification upon the characters of one organ or system of organs is to be condemned, and it has already been pointed out that HASSE was clearly too confident in hoping to classify the Elasmobranchii by the characters of the calcification of their vertebral centra, but the suggestion may here be offered to taxonomists of the future to look into the question of the relations between the Batoids and the Carchariid sharks. The inquiry might reveal other evidence of the possible origin of the Batoids from the base of the Carchariid stock.

In REGAN'S classification (**29**) the Batoids or Hypotremata are divided into four families, the Torpedinidæ, Rhinobatidæ, Raiidæ and Dasybatidæ. Accepting the eight-rayed star, of the type just described, as characteristic of the Rhinobatidæ, it is to be noted that it appears also in the Dasybatidæ (fig. 36, C, p. 391, and fig. 35, A, p. 391). In *Raia*, the sole representative of the Raiidæ examined, the vertical rays resemble those of the type just alluded to, while the horizontal rays, although similar, tend towards reduction (fig. 32, D and E, p. 387); the diagonal rays, if present at all, are irregular in their arrangement and growth.

Rhynchobatus and *Pristis* (fig. 31, p. 385) present a compact calcification of circular outline in transverse sections, but this is a condition which can be derived from the star in question by a process of broadening of all the rays until the intervening uncalcified parts disappear, a process the key to which is furnished by the vertebræ of the trunk region of *Rhinobatus* (fig. 29, p. 382). It is further to be noted that in the hindermost vertebræ of both *Rhynchobatus* and *Pristis* the outline is not circular, vertical and horizontal rays being recognisable (fig. 31, E); this condition recalls that found in the hinder caudal vertebræ of *Rhinobatus*, in which the diagonal rays are wanting, and only the vertical and horizontal rays are present (fig. 30, D, p. 383). There is no evidence in the vertebræ of *Rhynchobatus* and *Pristis* of any perichondrial contributions, and a study of young specimens of these genera points to the conclusion that the secondary calcification is wholly derived from the outer-zone cartilage (fig. 28, A, p. 382).

Included among the Dasybatidæ is a remarkable form, *Pteroplatea*, whose vertebræ are aberrant, in that they do not possess a recognisable double cone; the notochord has disappeared in the middle part of the centrum, and the calcification takes the form of a number of irregular nodules arranged in a circular manner just inside the membrana elastica externa (fig. 38, p. 393). This condition is evidently one of degeneration, and can have no value in taxonomy.

Although *Torpedo narce* exhibits an eight-rayed star, this does not exhibit the

same features as the star of *Rhinobatus* mentioned above; there are no diagonal rays, but the vertical and horizontal rays are duplicated (fig. 26, p. 379). The rays may grow out independently from the investing layer into the outer-zone cartilage, or two may arise as a single outgrowth, and fork subsequently (fig. 27, p. 380). In *Torpedo hebetans* the tendency for the rays to fork is less than in *Torpedo narce*; in *Narcine brasiliensis* it is much greater (HASSE, **15**, C, Plate 23, fig. 21). It is somewhat doubtful, owing to the early disappearance of the membrana elastica externa, whether the later growth of the calcified lamellæ is due to perichondrial activity (see p. 332). The centra of *Torpedo* and *Narcine* are readily distinguished from those of other Batoid fishes, and the conditions are such as to support a division of the Batoids into the Torpendinidæ on the one hand, and the Rhinobatidæ, Raiidæ, and Dasybatidæ on the other, as is proposed in REGAN'S classification.

SUMMARY.

In Elasmobranch fishes, the centrum—by which is understood that portion of the vertebra which is left after cutting away the projecting parts—is composed of two, sometimes three, kinds of cartilage, more or less calcified, namely: (1) sheath-cartilage; (2) arch-bases (the parts of the basidorsals and basiventrals nearest the notochord); and (3) intermedialia.

The sheath-cartilage, arising from skeletogenous cells that at an early stage migrate into the notochordal sheath through the membrana elastica externa from the basidorsal and basiventral cartilages, is early differentiated into inner, middle, and outer zones. The inner-zone cartilage is usually hyaline, and any calcification that may occur in it exhibits no regularity of texture; it is commonly traversed in the middle by the remains of the notochord, from which it is separated by the membrana elastica interna. The middle zone is composed of a close-celled cartilage with scanty intercellular matrix; the cells are fusiform, and their long axes are circumferentially disposed. The cartilage calcifies early, except in a few species, and the calcified structure constitutes the primary "double cone."

Calcification in the outer-zone cartilage occurs subsequently to that in the middle zone, and the calcified layers and lamellæ are consequently termed "secondary." Calcification of the innermost part of the outer-zone cartilage is of common occurrence, and produces an investment or "investing layer" (Belegschicht of HASSE) around the double cone, from which it may be distinguished by its rounder, more widely spaced cells, and its later origin. Further, whereas the calcification of the middle-zone cartilage proceeds simultaneously throughout the whole thickness of the middle zone, the calcification of the investing layer is progressive, the innermost part, in contact with the primary calcification, being the first-formed. The calcified lamellæ occurring in the outer-zone cartilage, whether radiating (*Cestracion*, fig. 4, A, p. 340) or concentric (*Squatina*, fig. 25, p. 377), are centrifugal

in their mode of growth; in the case of radiating lamellæ the new increments are applied to the external edges; in the case of concentric lamellæ the most external lamella is the most recently calcified. The investing layer may increased to such an extent as to produce a solid calcified mass around the double cone (*Pristis*, fig. 31, p. 385).

The third kind of cartilage, occurring in wedge-shaped masses, which it is proposed to term the "intermedialia" (Periostale Keile of HASSE), is developed later than the arch-cartilage and the sheath-cartilage (see p. 327); it increases centrifugally, usually from a growing zone just internal to the perichondrium. The initial cells of this growing zone arise from the perichondrium itself, just external to the membrana elastica externa. The cartilage of the intermedialia may calcify partially, with radiating lamellæ (*Lamna*, fig. 9, p. 356) or concentric lamellæ (*Cetorhinus*, young, fig. 13, p. 360) alternating with tracts of uncalcified cartilage; or the calcification may be complete (*Mustelus*, fig. 8, A, p. 354); or the intermediale may be reduced to a thin external layer (*Pristiurus*, fig. 16, C, p. 363). The matter is discussed at length in pages 327–331.

The present paper records the results of a study of the secondary and perichondrial calcifications in young and adult fishes belonging to 68 species and 44 genera. The results go to show that HASSE, in his great work, 'Das Natürliche System der Elasmobranchier auf Grundlage des Baues und der Entwicklung ihrer Wirbelsäule' (**15**), overestimated the importance of the disposition of the calcified masses and laminæ in the centrum as a taxonomic feature, and that, although his main groups of the Elasmobranch fishes are generally admitted to be, with certain reservations, natural groups, his classification was to a large extent arrived at by ignoring the vertebral characters in the sense in which he had described them. The centra of some of his Tectospondyli exhibit in transverse section a "star" quite as perfect as those found in his Asterospondyli (fig. 4, p. 340); and in the latter group he associated such forms as *Cestracion* and *Lamna* (fig. 21, A, p. 370, and fig. 9, B, p. 356) in which the "stars" are of different origins.

HASSE'S choice of the vertebra of *Pristiophorus* to illustrate tectospondyly in its most typical form (see p. 339) is singularly unfortunate in view of the discovery by REGAN that in a Pristiophorid from South Africa (*Pliotrema warreni*) the vertebræ have strongly marked radiating lamellæ (fig. 24, B, p. 375).

It is considered desirable to discard for general descriptive purposes the terms cyclospondylous, tectospondylous and asterospondylous. An attempt to introduce a new set of terms to describe the patterns of the lamellæ of the centra is not likely to succeed, because, while some types can with precision be designated by single words, others present great difficulties; and when in the same genus the patterns vary as much as they do in the several species of *Rhinobatus* (**15**, C, Plate 14, fig. 14;

Plate 15, figs. 27 and 41), it becomes clear that no great advantage is to be gained by the coining of new descriptive names for them. The matter is discussed at length on pp. 346–347.

It would appear that the calcified masses and lamellæ in the centra, developed in various positions in response to physiological demands for a strengthening of the cartilage for resisting stresses in certain directions, grow in definite directions, independently of the mode of origin of the cartilage in which the calcification is taking place. In *Stegostoma* (fig. 18, C, p. 366), for instance, the remnants of the membrana elastica externa show that while the inner part of each radiating lamella is developed in the sheath-cartilage, the outer part is developed in cartilage external to the sheath.

Similarly, in *Pristiophorus japonicus* and *Pristiophorus nudipinnis* there is a tubular calcification disposed lengthwise externally to the double cone, and free from it except at the two ends. A section through the middle of the centrum shows that this calcification is in the latter species a product of the sheath solely, whereas in the former (fig. 24, A, p. 375) there are, owing to the more sinuous course of the membrana elastica externa, four portions that occur in sheath-cartilage, while the other four are calcified parts of the arch-cartilages.

Again, in *Mustelus* it can be shown that, whereas the diagonal calcified lamellæ are usually in sheath-cartilage entirely, it sometimes happens that, owing to the failure of the sheath-cartilage to grow out sufficiently rapidly in the diagonal tracts, the calcification of the more distal parts of the lamellæ may occur in arch-cartilage (fig. 8, A, p. 354, right upper diagonal ray).

On the other hand, such disregard of the origin of the cartilage is not shown in the vertebræ of young *Cetorhinus* as compared with those of *Squatina* (fig. 13, p. 360), the concentric lamellæ of the former not being continued through the diagonal cartilages. The explanation of this is probably to be found in the fact that, while the intermedialia, in which the calcified lamellæ occur, are increasing by means of an external growing zone, the diagonal cartilages—basal parts of the basidorsals and basiventrals—are increasing by a general and uniform radial extension. In *Squatina* the growing zone, here of sheath-origin, is continuous all round.

It is clear from the difference in the origin of the concentric calcified lamellæ in the young *Squatina* and *Cetorhinus*, in the one case from sheath-cartilage, in the other from perichondrially produced cartilage external to the notochordal sheath—as also the difference in the origin of the radial calcified lamellæ in *Cestracion* and *Lamna* (fig. 4, A, p. 340, and fig. 9, B, p. 356), from sheath-cartilage and perichondrium respectively—that we are here dealing with cases of convergence, a homoplastic attainment of similar results in distantly related genera. It is equally clear from the resemblances, that there are certain mechanical advantages to be gained by the

calcification being in the form of concentric tubes in the first pair of genera, and in the form of radiating longitudinal lamellæ in the second pair. The question that arises from a study of these forms is—what mechanical principles are involved that have resulted in the evolution of concentric lamellæ in the former, whereas in the latter the necessary strength is attained by the development of radiating lamellæ? There is here indicated a promising investigation for anyone interested in the relation between mechanical requirements and the methods by which they are satisfied. Are there any resemblances, for instance, in the mode of flexure of the vertebral column associated with the habits of the fishes in question; are there any differences in the mode of attachment of the muscles to the vertebræ in the young *Squatina* and *Cetorhinus*, on the one hand, and the adult *Cestracion* and *Lamna*, on the other; is there any reason that can be ascertained for the concentric arrangement of the layers being continued throughout life in *Squatina*, whereas in *Cetorhinus* the later calcifications (fig. 14, A, p. 361) are mostly of the radial type?

There is nothing in the vertebral structure of *Squatina* and *Pristiophorus* to justify HASSE'S view—now largely discredited—that there is any close affinity between these genera and the Batoid fishes.

In some Batoids (*e.g.*, *Rhinobatus*, fig. 28, B, C, p. 382), the secondary calcification proceeds for a time by a gradual increase in the thickness of the investing layer until the surface is reached—in two places in the trunk vertebræ, in four in the caudal—and thereafter the increase in these two or four areas is by growing zones of perichondrial origin. The major parts of these lamellæ in the adult are thus external to the sheath, but their innermost parts are developed within the sheath-cartilage.

The Batoid fishes differ from the Sharks in the early disappearance of the membrana elastica externa; the part that longest remains recognisable is that between the two basidorsal cartilages. It is only in a few forms (*e.g.*, *Rhynchobatus*, fig. 28, A, p. 382) that any other relics of the membrane can be detected after the investing layer has begun to calcify. *Pteroplatea* is an exceptional form, showing remnants of the membrane in vertebræ of the adult (fig. 38, p. 393).

The results of the present investigation go to show that the distinction drawn by GADOW and ABBOTT (6) between chorda-centra and arco-centra is not so absolute as they claim. As regards chorda-centra, it is shown that in some Elasmobranch fishes (Lamnidæ) the part of the centrum of the adult that is developed from the notochordal sheath is small. On the other hand, in Amioid fishes, and possibly in some other forms with arco-centra not here considered, the centra appear to be derived in large part from skeletogenous tissue distinct from that of the arch-cartilages (p. 335).

In this paper the vertebræ of a number of species are figured for the first time, *e.g.*, those of *Scapanorhynchus owstoni* (fig. 11, p. 358), and *Pliotrema warreni* (fig. 24. B, p. 375); and figures are given of the vertebræ of species that have not been

adequately dealt with in previous papers, *e.g.*, those of *Raia* (fig. 32, p. 387), *Cestracion* (fig. 4, A, p. 340, and fig. 21, p. 370), *Cetorhinus* (fig. 14, p. 361), *Rhinobatus* (figs. 29 and 30, pp. 382 and 383). The figures of the vertebræ of late embryos are new, or are more correct representations than those already published. Special reference may be made to the figures of *Chiloscyllium* (fig. 17, E and F, p. 365), *Stegostoma* (fig. 18, C, p. 366), and *Rhinobatus* (fig. 28, B and C, p. 382).

In the course of the investigation there has been adopted a method of research that has hitherto been rather neglected, namely, that of studying the range and method of change of the pattern of the calcified laminæ of the centrum throughout the whole length of the vertebral column of the same individual fish, *e.g.*, in *Chiloscyllium* (fig. 17, A–D, p. 365), *Raia* (fig. 32, p. 387), and *Rhinobatus* (figs. 29 and 30, pp. 382 and 383).

The vertebræ of the great basking shark, *Rhinodon typicus*, do not seem yet to have been examined; it would be of considerable interest to ascertain how nearly they resemble those of *Ginglymostoma*, with which genus *Rhinodon* is associated by Regan (**29**, p. 745).

The vertebræ of *Orectolobus*, both of young and adult fishes, deserve further investigation if the material can be procured; also the vertebræ of late embryos of *Lamna* and *Alopias*.

Explanation of the Text-Figures.

The text-figures illustrating this paper are all partly diagrammatic; in some cases they are composite figures, drawn from two or three consecutive vertebræ. In the illustrations of Hasse's monograph (**15**) it is not easy, owing to a lack of diagrammatic treatment, to tell at a glance which parts are calcified and which are not, and a comparison between the various figures is thereby rendered difficult; the difficulty, moreover, is increased by the calcified parts being drawn darker than the cartilaginous parts in some of the figures, and lighter in others (**15**, D, *cf.* figs. 5 and 7, Plate 24; figs. 7 and 8, Plate 28; figs. 17 and 18, Plate 29).

In the present paper the texture of the various parts of the vertebræ is suggested, rather than represented in actual detail. In the figures that were drawn from dried vertebræ of adult fishes, the calcified parts are shown in black (fig. 9, A, p. 356); but in the drawings of sections of decalcified vertebræ cut with the microtome the look or appearance of the calcified parts is better secured by representing them by "broken" black areas, with a "scribbled" effect (fig. 8, p. 354). Lines of growth of calcified lamellæ, sometimes recognisable in the sections of the vertebræ, are not introduced into the figures. The lines in question are always more or less concentric around the primary double cone, whether they occur in radiating lamellæ, as in *Cestracion* and *Torpedo*, or in a solid mass, as in *Pristis*; and they have no direct bearing on the main thesis of the present paper. All calcareous accretion of

secondary origin is centrifugal, and there is thus no occasion to represent the lines or zones of growth in the figures.

The distinction between the primary cone calcification and the secondary calcification immediately around it, recognisable in the actual sections by a difference in texture or colour, is exaggerated in the figures, and in many cases a thin, white line is introduced to indicate the limit between the primary double cone and the investing layer. The white circle does not represent uncalcified cartilage; it is but a conventional means of drawing a distinction between the two kinds of calcification. If the septum in the middle of the double cone is calcified, it is diagonally shaded in the figures, in order to distinguish it from the double cone, with which it is really continuous at its edges (fig. 9, B, p. 356).

If on examination of a section of a dried vertebra with a lens the tesseræ appear as a continuous layer, they are shown by means of a thick black line, but if the individual tesseræ can be distinguished by the use of a lens, they are shown separately. Further, when the tesseræ are more granular in texture than usual, the granular effect is suggested by the tesseræ being drawn as "broken" or "scribbled" areas. Fibrous tissue is shown only in those cases in which its omission would be likely to create a wrong impression.

In the neural arches there frequently appear in the text-figures discontinuities of the cartilage, irregular, oblique, and sometimes present on one side of the figure only (fig. 11, B, p. 358). These represent the limits between the arch-components, the basidorsals and interdorsals, and the supradorsals (if such are present). They are introduced into the figures as they appear in the slices drawn, and no further interpretation of them is attempted. The motor roots of the spinal nerves commonly issue through the basidorsal cartilages in the sections selected, or else through a notch in the posterior border, and this affects the appearance of the figure by the introduction of a definite passage (fig. 29, A, p. 382), or, if the section includes only the margin of the foramen, a crowd of calcified tesseræ (fig. 34, A, p. 389). Since the present investigation concerns the centra almost exclusively, no special regard is paid to such points as these.

The broken line that indicates the position of the membrana elastica externa is, of course, greatly exaggerated. Except in the early stages of development, the remnants of this membrane have to be diligently searched for with a fairly high power of the microscope.

Explanation of the Lettering of the Text-figures.

A special lettering is employed in figs. 1, 3, and 6, and to these figures the abbreviations set out below do not apply.

bd, basidorsal cartilage, the principal component of the neural arch.

bv, basiventral cartilage, the principal component of the hæmal arch.

c, primary double cone, a calcification of the cartilage of the middle zone of the notochordal sheath.

cl, concentric calcified lamellæ in the outer-zone cartilage (*Squatina*, fig. 25, p. 377).

d, diagonal calcified lamella.

ee, membrana elastica externa, marking the outer limit of the cartilage of the notochordal sheath.

f, funiculus chordæ, the reduced notochordal remains in the middle of the length of the centrum.

g, growing zone.

i, island, or isolated nodule of calcified cartilage.

il, investing layer (Belegschicht of HASSE), a calcification of outer-zone cartilage immediately external to the double cone.

iz, inner-zone cartilage of the notochordal sheath.

l, intervertebral ligament.

m, solid calcified mass around the primary double cone (*Pristis*, fig. 31, B, p. 385).

n, remains of the notochord.

nr, passage for nerve-root.

oz, outer-zone cartilage of the notochordal sheath.

p, cartilaginous part of the intermediale, a structure of perichondrial origin, and not derived from sheath-cartilage, nor from arch-cartilage.

p', *p''*, calcified parts of the intermedialia.

r, radiating calcified lamellæ; they may be set independently upon the double cone (fig. 21, A, p. 370), or may radiate from a common base (fig. 4, B, p. 340).

s, septum, a transverse partition of fibrous, fibro-cartilaginous, or calcified tissue, usually traversed in the middle by the funicle.

t, tesseræ, granular calcifications just below the surface of the arch-cartilages; commonly in the form of a mosaic, but sometimes occurring as a continuous crust.

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IX.—*The Life-History and Cytology of* Synchytrium endobioticum (Schilb.), Perc., *the Cause of Wart Disease in Potato.*

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INTRODUCTION.

Wart disease of the potato was first reported from Hungary in 1896 by SCHILBERSZKY (43), who gave a brief description of the summer and the resting sporangia, named the organism *Chrysophlyctis endobiotica*, and included it in the *Chytridineæ*. He saw the discharge of the zoospores from the summer-sporangia, and he put forward the view that the zoospores were responsible for the further distribution of the organism through the tumour by the ability which he believed they possessed of boring their way through the walls of the host cell into the adjoining cells. The fungus probably existed in England many years before SCHILBERSZKY'S paper was written, but its presence in this country was not generally recognised. In 1902 POTTER (36) published a short paper on the organism. He there showed that resting sporangia, which had been kept dry during the winter, were able to cause the infection of tubers the next season. The distribution of the organism through the tumour he attributed to its division, when in a condition which he describes as plasmodial, and to the passage of the segments so produced through the walls into the adjacent cells.

For several years after POTTER'S publication appeared few facts were added to the existing knowledge of the disease, but in 1907 BORTHWICK (4) reported that leaves could be attacked as well as tubers. In the following year, SALMON (42) carried out a series of infection experiments, from the results of which he concluded that resting sporangia, after exposure for $1\frac{3}{4}$ hours to a temperature ranging from $-5°$ C. to $-6°$ C., could dispense with the winter dormancy and germinate at once. Shortly afterwards two notes appeared, one from the pen of JOHNSON (16) and the other from that of WEISS (49). Both succeeded in obtaining the germination of the resting sporangium, and, in addition, JOHNSON found that the zoospores liberated exhibited the usual characteristics of the Chytridian zoospore, while WEISS remarked upon the

rapidity with which they became amœboid. In December of the same year MASSEE (31), when exhibiting specimens of diseased tubers at a meeting of the Linnean Society, expressed the opinion that the organism belonged to the genus *Synchytrium*; but he based his statement upon the so-called epidermal nature of the parasite and upon the supposed presence of an enveloping membrane round the protruding contents of the germinating resting sporangium, both of which suppositions have since been shown to be incorrect.

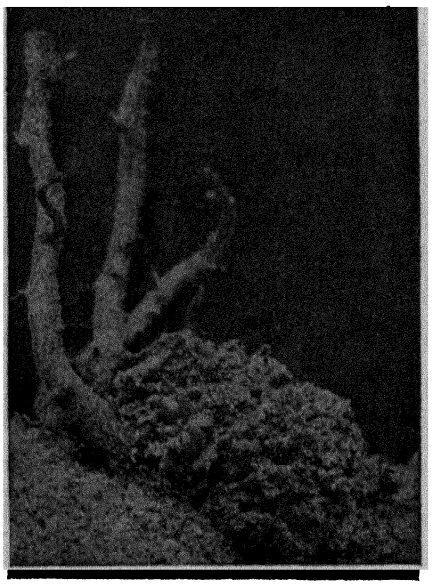
In the next year JOHNSON (17) questioned the validity of MASSEE'S statements, maintaining, on the one hand, that the organism may be deeply seated in the tissue of the tumour, and, on the other, that in the germination of the resting sporangium "the wall ruptures and the zoospores escape without any sign of the extrusion of a membrane." Not long afterwards appeared a paper by PERCIVAL (35), in which was made the first real contribution to our knowledge of the cytology of the organism. In the young resting sporangium the marked increase in size of the nucleus and nucleolus during the period of active growth, and the presence in the nucleus of an irregular structure called by PERCIVAL an "amœboid body," are briefly but plainly described. He believed that chromatin is transferred from the nucleolus to this amœboid body, and that about the time of the transference chromatin granules appear in the mesh of the cytoplasm, which then gradually breaks up into swarm-spores. These, PERCIVAL stated, were not always of the same size, and the possibility of an anisogamous fusion was suggested, although no union of any kind was observed. The method by which the secondary nuclei arose in the young sorus was believed to resemble that reported for the resting sporangium; the chromatin becomes associated with the linin in the nuclear vacuole, while the nucleolus loses its staining power and disappears. Chromatin meanwhile appears in the cytoplasm in the form of chromidia, and on the formation of vacuoles round these granules secondary nuclei are instituted. These small nuclei were found to undergo mitosis, and their behaviour was thus at variance with that of the primary nucleus. When the young sorus divides into its constituent sporangia the cleavage was believed to proceed from the periphery inwards. At the conclusion of the paper, on the basis of the morphological and cytological resemblance to members of the genus *Synchytrium* exhibited by the organism, PERCIVAL proposed that it should be placed in that genus under the name *Synchytrium endobioticum*.

The next noteworthy contribution to the life-history of the organism was published in 1912 by BALLY (1), who supported JOHNSON in his belief in the possibility of root infection of the host plant. In the resting sporangium this author ascribes the transference of chromatin from the nucleus to the cytoplasm to the migration into the cytoplasm of the granules which he observed studding the inner surface of the nuclear membrane. In the zoospores BALLY failed to find any trace of the change from the free-swimming to the amœboid condition such as had been reported by the earlier writers. With regard to the cytology of sorus, both macro- and micro-nuclei

were figured in the drawings, and mitosis of the primary nucleus, though not observed, was believed to take place. While BALLY preferred to retain the organism in the genus *Chrysophlyctis*, G. TOBLER (48), on the other hand, in a monograph of the genus *Synchytrium*, accepts PERCIVAL'S suggestion. For reasons to be given later, the organism in the present paper is also referred to the genus *Synchytrium*.

OUTLINE OF THE LIFE-HISTORY.

The fungus has no mycelium and at the reproductive period the whole body forms a sorus or a resting sporangium. In the spring the zoospores of the resting sporangium are liberated, and after a short period of activity they infect young tissue of the potato plant. The zoospore on entry passes to the lower end of the host cell, and there, after enlarging, develops a firm yellow outer, and a thin colourless inner, membrane (fig. 13). The protoplasm of the organism, while still in the uninucleate condition and enclosed in an expanding portion of the inner membrane, now passes through a small pore in the outer membrane into the upper portion of the host cell (figs. 30, 35). Repeated mitosis immediately takes place, and after five or six nuclear divisions the protoplasm segments into about five thin-walled sporangia, which together constitute the sorus (fig. 60). Further nuclear divisions occur and ultimately zoospores are formed. General division of the neighbouring cells of the host plant has meanwhile resulted in the formation of a tumour, while, in addition, the ring of epidermal cells in contact with the infected cell has grown up to form a rosette, whose projecting cells arch over the host cell lying at their base (figs. 2, 7). The sporangia when mature absorb water and enlarge rapidly, rupturing the soral envelope and the host cell wall, and so lie exposed to the exterior.



TEXT-FIG. 1.—Tumour on "Arran Chief." (× ½.)

The motile cells formed in the sorus have previously always been termed zoospores, but they may behave as gametes and fuse in pairs. The zygote resulting from the gamete fusion enters an epidermal cell of the potato plant, and there develops into a resting sporangium. By the division of the host cell, the resting sporangium becomes deeply placed in the tumour. Eventually the host cell dies, and its contents are deposited as a brown epispore upon the parasite (fig. 120). If fusion has been omitted, the facultative gametes ("zoospores"), on entering the plant, give rise to a sorus, and the zoospores developed in it either may or may not fuse. There is evidence that the treatment to which the maturing soral sporangia are subjected may decide whether zoospores or gametes are developed. In this paper the term

zoospore, for the sake of convenience, is retained as a general term for the motile cells formed in both soral and resting sporangia.

As it is impossible to determine the stage in the development of the sorus at which the organism passes into the reproductive phase, the term *prosorus* is applied to all stages between the completion of the rounding off of the zoospore (which takes place immediately after its entry) and the beginning of segmentation into sporangia.

Method of Investigation.

The most suitable varieties of potato for the purposes of inoculation were found to be Arran Chief, Mixed Early, and Midlothian Early. A reserve supply of diseased material was maintained by growing healthy tubers in soil from an infected field. Experimental work was conducted in the greenhouse, where the maintenance of a constant high temperature induced prolific growth in the tumour. It was essential that the development of the latter should be followed from day to day, and, although when tubers are planted sufficiently high in the soil, at least one malformation in each pot may be expected to reach the surface, where its subsequent growth can be watched, it was found more convenient to avoid the use of soil when the observation of a particular stage was desired. The most useful substitute for soil proved to be *Sphagnum*. The tubers were set to sprout under a light covering of the damp moss, but, at the first sign of growth in the eyes, sufficient *Sphagnum* was removed to leave the upper surface of the potato exposed. A drop of water containing active zoospores from the resting sporangium was then placed on one or two eyes in each potato, and for two days the plants were kept in a dull light and a saturated atmosphere. After this time they could be safely exposed to the normal conditions of the greenhouse. A few days after inoculation, small tumours were to be detected with the aid of a lens. The plants were then lightly watered once a day. At this stage the tumours bore only a few scattered sori, and, when stages in their development were required, the first, second, or even the third series of sori was permitted to discharge before portions of the tumour were removed for fixation. By that time the hypertrophy had attained a considerable size, and bore a plentiful supply of sori of all ages.

To induce the fusion of the soral zoospores, and so secure the formation of resting sporangia, the plants were not watered more often than once in four days. The object was to keep the soral sporangia undischarged and without water for at least two days after they had matured. On the addition of water, most of the zoospores, on being discharged, would fuse, and the greater portion of the new series would thus consist of resting sporangia. In the tumour, whose photograph is reproduced in fig. 1, the order of formation of reproductive bodies after control commenced was sori, sori, sori and resting sporangia mixed, sori, sori, resting sporangia. The resting sporangia do not germinate immediately, so, in the third generation of the above series, the zoospores of the sori were alone responsible for the production of the next

generation. Usually, not more than two series of sori and one of resting sporangia are formed in any particular region of a tumour, and in cases of natural infection in the soil large areas of the tumour may frequently be found, which seem to bear resting sporangia only. But, on careful examination, traces of a few ruptured sori can usually be found somewhere in the vicinity.

To secure stages of the entry of either form of zoospore, an abundant supply of slender shoots, less than $\frac{1}{4}$ inch long, is required. For this purpose clean tubers were planted in sawdust just beneath the level of the surface. When the shoots appeared above the surface, the plants were removed to a cool room, and water was withheld in order that growth might cease. Two days before the inoculation was to be made, however, they were thoroughly watered and replaced in the greenhouse. By the time they were required, each had produced great numbers of actively growing shoots of the desired size. A drop of water containing the zoospores was placed upon the apex of the shoot, and the plants were then kept in a saturated atmosphere until the time of fixation.

The fixative used was FLEMMING'S strong solution, diluted with an equal quantity of water. The reaction of the organism to stains varies widely, according to the stage of development. HEIDENHAIN'S Iron-alum-hæmatoxylin, FLEMMING'S Triple Stain, and BREINL'S Triple Stain, are all excellent for the staining of any particular stage. But only FLEMMING'S Triple Stain (or its simpler form, Gentian violet and orange G) can be relied upon to give uniformly good results when the examination of all the stages occurring on a slide is contemplated.

MORPHOLOGY OF THE PROSORUS AND SORUS.

The colour of a tumour growing above ground varies from white to deep green, according to the intensity of the illumination to which it has been exposed, and, if the potato belongs to a variety which normally has purple markings, the edges of the surface irregularities will probably be correspondingly coloured. By means of a lens, the presence of maturing sori can readily be detected, owing to the paler shade and minute granulation of the surface which bears them. Both of these characteristics are due to the presence of the projecting and highly refractive rosette cells surrounding each sorus.

In a surface view of the epidermis of the tumour under a low power objective, the young prosorus is to be distinguished as a densely granular sphere, with a thin, colourless wall, and a lighter area in the centre, where the nucleus lies. The host cell is much enlarged below, and little protoplasm can be detected in it. When the organism is quite young, the cells of the tumour adjoining the host cell do not project above the level of the surface, as may be seen in the three parasites to the left of the empty rosette in fig. 8. The wall of the prosorus gradually thickens, and becomes light golden-brown in colour. The same colour may soon be observed in the wall of the host-cell also, but in this case the change is probably due solely to the

deposition of the contents of the host cell upon it. Usually, only one prosorus occurs in each host cell, but two are not uncommon (fig. 29), and, on one occasion, four host cells, almost adjoining one another, were found to contain two, three, three, and four prosori respectively. Occasionally, prosori and resting sporangia lie side by side in the one cell.

The migration of the contents of the prosorus into the upper portion of the host cell, which does not take place until after the membrane has assumed its golden colour, may be slightly oblique (fig. 30), but the general direction is always upwards, the vacated membrane being invariably left below. The discharge is completed in about four hours and in favourable material the process can be watched under the microscope. The rate of movement of the protoplasm is at first very slow, but it increases rapidly as the process continues. (For stages of the migration see figs. 29–35.) The epidermal cells adjoining the host cell begin to enlarge about the time of the migration of the prosoral contents, and thenceforth they keep pace with the expansion of the host cell (fig. 12). They are refractive, turgescent cells and when viewed from above are seen to be arranged in a radial fashion round the depressed host cell at their centre (figs. 3, 4, 5). As they enlarge still further their free ends curve slightly inwards, arching over the host cell and forming a natural trap for the retention of moisture upon its surface. At maturity they are divided transversely by walls formed parallel to the surface of the tumour, and the width of the whole rosette is increased through the epidermal cells lying at the periphery undergoing a slight and relatively late enlargement (fig. 7). Cleavage of the prosorus takes place soon after its migration, and as the maturing sporangia absorb water and swell the host cell is subjected to great pressure. The cells of the host tissue immediately beneath it have meanwhile undergone repeated division in the direction parallel to the surface of the tumour, and as they also absorb water and swell at the same time as the sporangia, the upper wall of the already distended host cell bursts (figs. 67, 71). As the result of the rupture the upper sporangia are usually jerked out, but the others remain in the host cell and there effect the discharge of their zoospores (fig. 72*a*). The lower sporangia are the latest to mature and their zoospores may be liberated as late as thirty-six hours after the sporangia have become free. Those of the upper sporangia, on the other hand, are usually discharged immediately after the rupture of the host cell. After the rupture the crumpled remains of the outer wall are left as an irregular brown ring round the top of the cell (fig. 8).

The Prosorus.

The Cytology of the Prosorus.

As soon as the entering parasite lies wholly within the epidermal cell it assumes the spherical form which is regarded in this paper as the beginning of the prosoral stage. It then begins to pass towards the lower end of the cell (fig. 9). The means by which the parasite moves is somewhat obscure. There is certainly no indication of the

assumption of an amœboid form, the existence of which has been claimed by some previous writers. A perfectly spherical form is preserved throughout the period of movement and probably the organism is carried passively by the protoplasm of the host cell. The body of the prosorus at this stage consists of a few delicate strands of protoplasm radiating from the nucleus to the periphery and separated by large vacuoles. At the time when the downward movement in the cell begins the nucleus may not yet have regained its spherical form after the compression to which it was subjected in its passage through the wall (fig. 10*a*). It is now in a transitional stage between the condition of the nucleus of the zoospore, in which there is a clear central vacuole and the chromatin is confined to peripheral granules (fig. 74*b*), and that of the more mature prosorus, where the centre is occupied by a single large nucleolus and peripheral granules are absent or inconspicuous (fig. 19). The exact nature of this transitional stage is somewhat obscure. At this stage it is difficult to detect any structural differentiation in the nucleus. The outline is a little irregular and there are indications at its periphery of the original granules of the nucleus of the zoospores (fig. 10*b*). Staining material, however, is being formed so rapidly that soon all that can be observed is a dark homogeneous granule of spherical form (fig. 10*c*). A small clear area next appears round the granule (fig. 10*d*) and there is thus formed the normal vacuolated nucleus which persists until the first nuclear division occurs. Similar stages of nuclear development are to be followed more clearly in the parasite developing from the zygote (p. 445).

When the prosorus is established in its final position it commences to enlarge at an extraordinary rate and the nucleus, which increases in size even more rapidly, soon forms a strikingly large object in the centre of the organism. During the enlargement the amount of protoplasm has increased to a marked extent and the earlier arrangement in radiating strands is gradually replaced by a reticulum of uniformly small mesh (compare figs. 9 and 10).

The parasite has usually reached the lower end of the host cell before it causes any change in the size of the cell. Zoospores have regularly been found to congregate particularly on the surface of the tissue of which the epidermal cells are undergoing rapid division; in any group of such actively dividing cells those whose nuclei are actually engaged in the process of mitosis seem to be especially liable to infection. The effect of infection upon these host cells becomes evident earlier and usually is more marked than upon those whose nuclei are in the resting condition at the time of infection. The first effect produced by the presence of the parasite consists of a rapid enlargement, chiefly in the downward direction, so that the host cell becomes a large pyriform sac with its outer wall remaining for some time unaltered in size (fig. 11). By this time the protoplasm is reduced to a thin layer covering the parasite and the walls of the cell. The host nucleus, however, increases considerably in size, but except when compressed by the expanding prosorus it suffers no distortion until just before its death, which occurs somewhat later.

The structure of the nucleus of the prosorus now becomes more complicated as the result of a series of discharges of the substance of the nucleolus into the vacuole of the nucleus. The chromatin thus discharged gradually dissolves in the vacuole leaving behind a linin matrix. Before entering upon a more detailed description of the nucleolar discharges, however, the general appearance of the prosorus and host cell during that process will be briefly described.

In fig. 12 is shown an enlarging but still young prosorus. The nucleus is conspicuously large but the greater portion of it is occupied by a vacuole. The heavy nucleolus lies suspended at the centre by a few delicate linin strands, and upon the latter and upon the nuclear membrane are the remains of the chromatin previously discharged from the nucleolus and now undergoing solution in the vacuole. Scattered here and there in the reticulum of the cytoplasm are minute chromidia, which make their appearance only after the process of nucleolar discharge has begun, and bounding the whole is the thin, almost invisible membrane of the organism. The enlarged nucleus of the host cell is resting upon the parasite and its deep staining reaction indicates that its disorganisation is beginning. The protoplasm of the host cell is now reduced to an extremely thin layer upon the wall of the parasite. On the right-hand side the epidermal cell in contact with the host cell has followed the upward development of the latter and has subsequently become divided by transverse walls. This represents an early stage in the upward growth of the ring of epidermal cells surrounding the host cell which culminates in the formation of the rosette. A slight enlargement can be detected even in the second cell from the host cell, as the third, of which only a small portion is depicted, lies at the level of the surface of the tumour. The width of the outer wall of the host cell represents the original width of the cell when infection took place. On the left-hand side there are no rosette cells, and the wall of the host cell is straight, the explanation of both these relations lying in the fact that the adjacent cell on that side has also been infected; for it has invariably been found that when two infected cells adjoin, the common wall is kept straight by mutual pressure and the two organisms share the one rosette. Should a single uninfected cell intervene, however, its presence is sufficient to ensure the formation of an individual rosette for each host cell. The host cell figured has probably reached its full size, but the prosorus will continue to enlarge until it fills the lower portion of the cell (fig. 13). In this figure is shown a more mature prosorus where the contents are about to pass into the host cell. The point in the membrane through which the discharge will take place lies at the centre of the portion in contact with the finely granular protoplasmic disc in the upper region of the organism. The membrane itself, which has attained considerable thickness, is, in living material, clear in substance and golden in colour. It consists of two layers, the outer of which is thick and coloured, while the inner is thin and hyaline. The process of nucleolar discharge is now almost completed, and the nucleus exhibits the appearance typical of the migrating prosorus. The nucleolus rests upon the nuclear

membrane, while rising from it and stretching across the nearly homogeneously filled vacuole are a few long, distinct, linin strands. This is really an early stage of the prophase of the primary mitosis, the later phases of which will be completed when the nucleus is reconstituted after the passage of the prosorus into the upper part of the host cell has taken place. The number and size of the chromatin granules in the cytoplasm have markedly increased as the result of the solution of the chromatic substance in the nuclear vacuole, and its subsequent condensation into chromidia in the extra-nuclear region of the organism. The host protoplasm lying between the parasite and the walls of the cell exhibits the affinity for chromatin stains common to disintegrating cells. This is an early sign of the changes which finally result in the deposition of the protoplasm upon the prosorus as an epispore similar in colour and general appearance to the membrane of the parasite. Through the agency of this layer the envelope of the prosorus eventually becomes firmly welded to the lower portion of the host cell in which it lies.

The Discharge of Chromatin from the Nucleolus.

The process of nucleolar discharge sets in so early in the development that prosoral nuclei in which it has not yet begun are comparatively rare. Such a nucleus, however, is shown in fig. 14. It is quite small and its structure is simple, consisting only of a central nucleolus suspended by a few radiating linin strands in the centre of the large vacuole. Before the contents of the prosorus pass upwards in the host cell three discharges of the nucleolar substance takes place, but as the first is insignificant in amount and similar to the second in method a description will be given of the second and third only.

The Second Discharge.—The nucleolus in the stage immediately preceding the second discharge is large and spherical. It stains uniformly except for the small vacuole which is the result of the material lost in the first discharge (fig. 14*a*). This vacuole, however, usually disappears before the second discharge takes place. As a preliminary to this discharge, the chromatin is found to stain more deeply at one or two points on the surface of the nucleolus. These areas become gradually raised, and finally they are pushed up in the form of globular buds above the surface of the nucleolus (fig. 15). Their substance is then opened out, both upon the linin strands already present in the vacuole, and independently, in the form of dark threads of irregular outline interlacing with one another on the side of the nucleolus on which the globule was formed (fig. 16). In the earliest stage these jagged threads stain uniformly, and seem to be wholly composed of chromatin, but before they have been long in the vacuole a change takes place in their appearance, and their two-fold nature is revealed. All portions of the threads alike become paler as the result of the chromatin solution which sets in, but where the irregularities are large the process takes longer, and the narrower portions of the thread in consequence may be in an almost colourless state, while the irregularities

are reduced only to condition of isolated granules and still retain a certain proportion of their chromatin. After partial solution, therefore, the effect produced is that of linin strands beaded with minute chromatin granules (fig. 17). There is wide variation in the amount of nucleolar substance emitted at this discharge. It usually passes out in one large globule, but several smaller ones may be given off at the same time. As the substance of each is distributed the nucleolus is left with vacuoles corresponding in number and size to the globules emitted (figs. 18*a*, *b*). But, apart from the major periodic discharges, when the organism is in its normal state of active growth the nucleolus continues to give off small independent globules, so that when the first discharge has once begun it is exceptional to find a nucleolus without globules upon its surface in some stage of formation. Thus, in fig. 16, the second discharge has only just taken place and the globules upon the surface are of the occasional nature described above.

As the chromatic constituent of the threads disappears the achromatic matrix upon which it was supported is gradually revealed. As time passes the now colourless strands are widened, their edges become diffuse, and the whole substance is gradually distributed more or less evenly through the vacuole as an extremely fine network. The longer the period between this discharge and the next the more uniform is the distribution of the linin matrix through the vacuole. Two stages in the process may be seen in the nuclei of figs. 18*a* and 18*b*, and in the first in addition the linin may be traced to its point of attachment to the mouth of the large vacuole in the nucleolus. The size of the nucleus has so far been regularly increasing, but the maximum is attained during this discharge when the diameter of the nucleus may be as great as 25 μ. After this stage, however, the nucleus gradually contracts until at the migration of the prosorus the diameter may not be more than about two-thirds of the maximum size.

The Third Discharge.—In an active nucleus the loss of nucleolar substance due to the second discharge will be replaced by fresh chromatic material before the next discharge begins, and the globules of the latter will once more develop upon a uniformly dark nucleolus (fig. 19). In other cases the globules are formed upon a vacuolated nucleolus, and this condition is associated with incompletely dissolved chromatin in the vacuole (fig. 20). In the nucleus of this figure the numerous globules characteristic of the third discharge have already been formed, and are now about to be discharged. Their dark colour presents a sharp contrast to the almost colourless nucleolus upon which they rest, and the latter has apparently given up the greater portion of its chromatin for their formation. During the accumulation of chromatin substance for the formation of the globules of the third discharge the nucleolus moves from its central position to the nuclear membrane.

In fig. 21 the third discharge is in actual progress and the chromatic substance is being transferred from the globules to long heavy strands. It is in a stage such as this that the function of the globules as distributing centres may be most clearly

recognised. The surface view of a nucleolus at the same stage is shown under higher magnification in section (*a*) of the same figure, and it will be seen that, although the nucleolus is honeycombed with vacuoles and contains very little chromatin, the globules have suffered no reduction in concentration but only in size. In the homogeneous nucleoli of the younger prosori the substance of the globule is usually drawn from the peripheral region, and the size of the resulting vacuole does not greatly exceed that of the globule formed, but in the older, vacuolated nucleoli, in addition to the granules rising near the surface, others are formed deep within the body of the nucleolus. These apparently pass up to the surface through the connecting system of vacuoles, and there either unite their substance with that of the peripheral granules or give it off independently into the vacuole. Owing to the weak concentration of the chromatic element in the old nucleolus, a large area is drawn upon in the production of a granule of the requisite density, and the relative sizes of granule and vacuole in consequence present a marked contrast to those of the younger nucleoli (*cp.* figs. 26*a* and 26*b*).

It is a characteristic feature of the third discharge that the chromatic threads in which the nucleolar substance is distributed in the vacuole are not arranged in an irregular interwoven fashion as in the second discharge, but are limited in number and extend in more or less regular sweeping curves from the nucleolus across the nucleus to the surface of the membrane opposite (fig. 21). By this time the linin arising from the previous discharge is so evenly distributed through the nuclear cavity that trace of the reticulum is almost completely lost, and in the figure mentioned only in a small region near the periphery at the left-hand side can the reticulate arrangement still be seen. In the final condition the whole background of the vacuole consists of homogeneous linin, and no reticulum remains. At this stage the nucleolus sometimes becomes flattened into a disc-shaped structure, and is closely adpressed to the membrane (fig. 23), but this is by no means an invariable rule, the original shape being frequently retained to the end.

The solution of the chromatin, perhaps owing to the altered condition of the vacuole, proceeds slowly after this discharge, and, in consequence, the method of transference from globule to strand, and the stages of solution when upon the strand can be followed more easily in this last discharge. Fig. 22 shows a small portion of a nucleolus, and at the surface is a globule which is transferring its substance to the two linin threads attached. As will be seen from the isolated positions of the granules already upon the threads solution has commenced, but transference of the substance is still proceeding, and under natural conditions it would have continued until the supply was exhausted. As the nucleus matures the width of the strands is gradually reduced, the unevennesses upon their surface are lessened and eventually removed, and they become uniformly slender and regular. In the latest stage, when the chromatin has completely disappeared from them, they persist as delicate but perfectly distinct strands spanning the nucleus.

In the early stage of the discharge, in addition to the long strands, there are frequently a number of shorter ones, but these in time disappear, and finally only five long ones remain (fig. 24). In the nucleus reconstituted after the migration of the prosorus the nucleolus is small and homogeneously dark (fig. 34). The contraction in volume, with the concentration of chromatic substance it involves, may take place before or during the migration. A peculiarity of the third discharge is that the larger irregularities upon the threads do not always remain attached to them during their solution, but frequently become separate, and then are to be seen as dark isolated granules lying usually near the periphery of the nucleus. This severing of portions of the chromatic element from the strands is probably largely responsible for the eventual smoothness and delicacy of the latter (fig. 24*a*). In this figure, contraction of the nucleolus has already occurred, although the movement, which has begun in the cytoplasm of the parasite, has not yet been communicated to the nucleus.

In the majority of cases under normal conditions the nucleus slowly undergoes these successive changes, and reaches and duly completes the final stage before the migration of the protoplasm occurs. But the description given above of the final appearance of the nucleus holds only for the usual cases in which delay has occurred between the second and third discharges, and in which the stages of the latter discharge are actually completed. At the completion of the second discharge, however, the prosorus is extremely sensitive to variation in external conditions, and its behaviour varies widely according to the regularity of the water supply in particular. Under the abnormal temperature of the greenhouse, when there is a constant supply of water, there may be no delay between the discharges, the stages of the third may be quickly passed over, and the prosorus may migrate before this discharge is actually completed. In this case, the outer envelope of the organism will be comparatively thin and still pale in colour when migration takes place. The nucleus of a prosorus which has undergone this rapid development is shown in fig. 25. The absence of delay between the discharges and rapidity in the carrying out of the third may be deduced from the fact that the linin formed in the earlier discharge has not yet completely changed from the interwoven to the reticulate condition, much less has it acquired the final uniform appearance found in prosori developed at a lower temperature. Moreover, the later stages of the third discharge will not be finished either slowly or completely before the nucleus begins to move, for the cytoplasm of the organism is already commencing to migrate (not shown in the figure). This, however, is a case of exceptionally rapid development. As a rule, a distinct pause occurs between the two discharges, and the envelope of the organism at the same time becomes thick and deep yellow in colour. Then, once the third discharge has begun, the prosorus migrates, either early or late according to the water supply, and the appearance of the nucleus at the time of migration thus depends upon the same regulatory factor.

The Passage of the Prosoral Contents into the Host Cell.

Shortly before the passage of the prosorus begins, the protoplasm immediately beneath the place where the channel will be formed becomes conspicuously dense, its vacuoles disappear, and its chromatin granules are dissolved (fig. 27). In material which has been fixed in FLEMMING'S solution and left unbleached, this portion alone of the cytoplasm appears light in colour and free from granules, standing out in sharp contrast to the mass of vacuoles deeply stained with osmic acid that masks the cytoplasm elsewhere. The protoplasm round the upper portion of the nucleus also gradually becomes condensed, and forms a small triangular cap with the apex pointing in the direction in which the discharge will take place. The cytoplasm at the centre of the superficial dense area, while still covered by an expanding portion of the thin inner lining of the envelope, now soon pushes as a minute projection through the heavy outer membrane into the clear space of the host cell beyond (fig. 28*a*). Except for the small portion of the inner lining of the envelope which lay beneath the pore, and which continues to expand as the mass of the prosorus emerges, the inner lining as a whole remains in its original position within the envelope. Under the pressure of the out-flowing protoplasm the edge of the envelope at the pore turns first up, and then slightly back as the contents expand over the free surface of the envelope (figs. 28*a*, *b*). When the projection is still quite small, the upper portion of the nucleus begins to elongate, but the basal region remains for a little time practically unaltered so that the nucleus becomes pyriform in shape (fig. 30). As the apex of the nucleus extends upwards it is preceded by an elongated cytoplasmic strand, which reaches towards the pore and consists of the substance of the cap previously surmounting the nucleus. The preliminary stage of migration takes a comparatively long time, but once the nucleus begins to move, the discharge becomes more rapid, and the passage of the nucleus itself is quickly accomplished. The elongated apex of the nucleus continues to be drawn out until it reaches and passes through the pore, but as the major portion has probably not yet moved from the original position, the nucleus at this stage may be extended over more than half the width of the prosorus (fig. 31). During this elongation the nucleolus usually lies in the posterior end of the nucleus, but it may lie further forward, and, in exceptional cases, it has been found in the narrow anterior region. In most migrating nuclei it is a conspicuous object. It may be already reduced in size, and its contents condensed and darkly stained. More often, however, it is large, has many vacuoles, and stains lightly. If it lies in the posterior end of the nucleus, the fine linin threads, which are usually five in number, will be attached to it at one end, and remain extended before it in long sweeping curves during the passage. When the nucleolus is placed in the anterior end, however, the linin threads are drawn after it. Probably a few chromatin granules, which have previously been detached from the linin threads, are to be seen on the inner surface of the nuclear membrane, and sometimes they undergo

budding during and just after the passage of the nucleus. The vacuolar region is completely filled with the fine homogeneous substance which arose as the dissolution-product of the material of the nucleolar discharges. The nucleus narrows before reaching the pore, and becomes still more reduced in diameter as it passes through it. The vacuolated condition of the nuclear cavity is never retained after the reconstitution of the normal form of the nucleus.

As soon as the pointed apex of the nucleus has emerged from the aperture of the pore it begins to swell into a spherical shape, but at the same time it continues to move forward (fig. 32). The direction of movement is maintained in the same straight line, and the nucleus finally takes up its position close beneath the thin membrane of the prosorus at a point opposite the pore (fig. 34); the last stages of the nuclear passage (fig. 33) are completed extremely rapidly, but one or two cases have been found in which, while the major portion of the nucleus has resumed its spherical form, the posterior region is still drawn out towards the pore behind it.

At the beginning of the migration of the prosorus large vacuoles arise at the periphery of the protoplasm in the original envelope as it retreats from the membrane (fig. 34). As the body continues to emerge from the pore the vacuoles become larger and larger until finally there remain only a few delicate strands which reach across the width of the envelope and unite at the pore (fig. 35). Stages in the retreat of the protoplasm are shown in fig. 36*a–c*. After the completion of the passage, and even later when several nuclear divisions have taken place, a few odd strands of protoplasm may still be detected in the vacated envelope. The migration is apparently brought about by a rapid absorption of water, and the consequent formation of a series of vacuoles round the periphery of the prosorus under the pressure of which the contents are extruded through the envelope. It is not unusual at the completion of the discharge to find a large vacuole extending from the nearly empty envelope to the centre of the extruded mass of cytoplasm (fig. 35). The vacuole there soon becomes broken up into numerous smaller vacuoles, and they in time are evenly distributed throughout the prosorus, and are then no longer distinguishable from the normal vacuoles of the cytoplasm.

Mitosis in the Prosorus and Sorus.

A considerable amount of work has already been published upon the method of nuclear division in the prosorus and sorus of other species of *Synchytrium*. *S. taraxaci* and *S. decipiens* are the two which have been frequently investigated, but even in their case investigators are agreed neither upon the universality of the occurrence of mitosis nor upon the details of such nuclear divisions, whether amitotic or mitotic, as they describe. All the species, whose nuclear phenomena have been carefully examined, belong to the group *Eusynchytrium*, to which group *S. endobioticum* also belongs. In spite of the disparity in the results so far obtained, it seems not improbable that in the prosorus and sorus not only in the group *Eusynchytrium*, but

in that of *Pycnochytrium* also, further research will reveal the existence of a uniform method of nuclear division and confirm the growing belief that that method is mitotic.

The first worker to examine the phenomena of nuclear division in the sorus was DANGEARD (6), who, in 1890, stated that in *Synchytrium taraxaci* the primary division (*i.e.*, of the original nucleus, in contrast to the secondary or succeeding divisions) was always amitotic, but that at least some of the succeeding divisions were definitely mitotic. His work was confirmed by that of ROSEN (37), who believed that amitosis occurred exclusively in the earlier divisions while typical mitosis was gradually evolved during the later divisions. F. L. and A. C. STEVENS (46), working together upon the American species *S. decipiens*, came to the conclusions which, on the whole, were at variance with those of DANGEARD and ROSEN. Mitosis they maintain is the normal form of the primary division, but they qualify their statements by adding: "We may here indicate, also, that in affirming that the primary division may be mitotic, we in no way set aside the possibility of its being sometimes, even frequently, amitotic." Of the method occurring in the succeeding divisions they give no information, but GRIGGS (11), using their material, concluded that in these divisions both mitosis and amitosis were to be found. The cases of amitosis occurred in the divisions that immediately succeeded the primary, and two forms of it were recognised—nuclear gemmation and heteroschisis—but later in the development of the sorus mitosis was found to be the only method of division. The nuclei which arose by amitotic division GRIGGS believed could afterwards undergo mitosis, but no variation was detected in the number of chromosomes of the later divisions. The interpolation of amitotic division did not apparently present any difficulty to him in connection with the question of heredity, for he adds, "there is no morphological or material continuity of the chromosomes from generation to generation of nuclei; but the chromosome number is a physiological constant, like the other hereditary characters of the species."

KUSANO (22), in an excellent paper upon the Japanese species *S. puerariæ*, maintained that normally all the divisions, primary and secondary alike, are mitotic, but he admitted he had at times found clusters of nuclei adhering together which he believed had probably arisen through some process of amitosis. The presence in the sorus of nuclei of unequal size has been noticed by all the writers and in every case their origin is attributed to a previous occurrence of amitosis.

In the present species PERCIVAL (35) has seen and figured the metaphase of small secondary nuclei, but though he failed to find the primary nucleus in division he was evidently inclined to the opinion that the method was amitotic for he states: "No primary nucleus was found to undergo recognisable mitotic division, but undoubted mitosis occurs in the minute secondary nuclei. In this respect my observations upon this organism agree with those of DANGEARD (6) and ROSEN (37) who noted the gradual evolution of typical mitosis in the later divisions of *Synchytrium taraxaci*."

BALLY (1) described the secondary divisions in *S. taraxaci* as definitely mitotic, and although he did not succeed in finding the primary division he concluded that it also was mitotic. In the primary nucleus, however, he describes a passage of chromatin granules from the nuclear vacuole into the cytoplasm, which if confirmed, would but add another form of amitosis to the numerous types already described for *S. taraxaci* alone.

With the publication of a paper upon *S. taraxaci* by RYTZ (38) in 1917, however, the aspect of the question of nuclear division in that species was completely changed. In this investigation it is maintained that mitosis is the only method of division of both the primary and the secondary nuclei.

All the investigators who succeeded in finding cases of mitosis agree that the spindle is intranuclear in origin and that prior to the formation of the chromosomes a large portion of the substance of the nucleolus is given off into the nuclear vacuole. Concerning the time of the disappearance of the nuclear membrane, however, there is wide variation of opinion.

Thus in spite of the thirty years which have elapsed since DANGEARD'S paper was published, no definite conclusion has been formed concerning the nature of either the primary or the secondary divisions. On the whole, however, mitosis has come to be regarded as the normal method, and the accepted range of its occurrence has been extended to include the division of the primary nucleus, while concomitantly amitosis is now considered a somewhat exceptional method of nuclear multiplication in the genus and ranks as a subsidiary phenomenon only.

The Division of the Primary Nucleus.

Prophase.—Although the beginning of the prophase of the first nuclear division is difficult to determine, it may be considered to begin with the setting in of the processes of the third (last) nucleolar discharge, as it is at that time that the changes are initiated that lead directly to the formation of the spindle and chromosomes. By the time migration of the organism commences the surplus substance of the nucleolus has been given off, the vacuolar region is in the dense homogeneous condition characteristic of it during metaphase and anaphase, and the fine linin threads which will constitute the spindle are already formed. Actually there is no break between the pre-migration and post-migration stages of the nucleus. The migration of the whole prosorus may be completed in four hours; the nucleus moves early, and by the time two-thirds of the cytoplasm have passed from the envolope, it will have reached its final position in the extruded portion, have regained its original shape, and be preparing for the formation of the spindle of the primary division (fig. 34). Two nuclear divisions usually occur before the cytoplasm has passed out completely, that is, since the rate of movement of the second half is more rapid than that of the first, in less than two hours (fig. 35). If the early divisions in other species of *Synchytrium*

take place at a similar pace it is not strange that difficulty has been experienced in securing stages of the primary division.

The appearance of the reconstituted nucleus closely resembles that of the nucleus before migration, except that the former is slightly smaller (fig. 34). It is oval in shape and lies close to the free surface of the prosorus with its longer axis at right angles to the direction of migration. The cap of dense cytoplasm which lay upon its upper surface before migration and passed in advance of it when movement began, becomes spread out close to the surface of the prosorus in the neighbourhood of the nucleus. The lower side of the nucleus is sometimes slightly flattened, perhaps owing to the pressure of the cytoplasm which is still flowing up beneath it. The nucleolus lies in an excentric position close to one of the ends of the nucleus. It is reduced in size and always stains deeply. The free chromatin granules sometimes visible in the nucleus at the beginning of migration have usually disappeared by the time it takes up its final position. The nuclear membrane is extremely delicate, but it separates sharply the fine homogeneous ground-substance of the nucleus from the somewhat dense, non-vacuolated cytoplasm immediately surrounding it.

After the reconstitution of the nucleus one or two buds appear upon the surface of the nucleolus, and it is believed that the chromosomes are formed from one of these on its separation from the nucleolus (figs. 39, 40). The five linin threads which before and during migration extended in long open curves from the nucleolus are now contracted somewhat towards the latter and form a loose coil of interwoven threads, the individual strands of which, however, remain distinct (fig. 37). This stage soon passes and they are drawn out again until they lie close, and almost parallel, to one another through the agency of extremely fine, only just visible linin threads, which in specially favourable material may be seen attached to ends of the strands, joining them to the nuclear membrane (figs. 38, 39). The nucleolus now becomes slightly separated from the strands, and they become finer and finer the nearer they approach one another (fig. 40). At the stage when they are almost touching to form the spindle five minute oval chromosomes can be detected upon them in the equatorial region. The actual origin of the chromosomes was not observed, but as the nucleolus persists intact but reduced in size after the formation of the spindle, and, as in addition, no free chromatin granules (save occasional disintregrating particles on the inner surface of the membrane) can be found in the nucleus at the time, the conclusion has been drawn that the chromosomes originate probably from one of the latest nucleolar buds given off after the reconstitution of the nucleus.

F. L. and A. C. Stevens (46) believed that in *S. decipiens* the substance of globules found in the nuclear vacuole was drawn out into sharply intersecting, short linin threads, which later coalesced, to form the strands of the spindle. The dense halo of granular substance noticed round the latter at metaphase was attributed to a swelling and gelatinisation of the nuclear membrane, and the possibility of its being the after-product of an intranuclear element, as in *S. endobioticum*, was not con-

sidered. The chromosomes, which they figure as long narrow structures, were said to be only four in number, in contrast to the five of the present species. The only other description of a primary mitosis is that given by KUSANO (22) for *S. Puerariæ*. In this species, the dense background surrounding the newly formed spindle was believed to represent a dissolution product of the nuclear elements. In the case of *S. endobioticum*, however, we may go a step further, and state that the element in question is the linin residuum of the chromato-linin globules discharged from the nucleolus, and that in many cases it is a conspicuous feature some time before the spindle is formed. The spindle itself, KUSANO believes, is usually constituted from the residue of the nucleolus, but he gives no explanation of its origin in those cases in which the nucleolus was found to persist throughout the later stages of mitosis. The chromosome number is stated to be definitely five, and thus it agrees with that of the present species. In one particular, however, that of the time of the disappearance of the nuclear membrane, there is not complete accord between KUSANO'S description and the behaviour of *S. endobioticum*. In the former it disappears before any sign of spindle formation is to be detected, whereas in the latter it persists until metaphase—a condition which KUSANO acknowledges to be normal for all succeeding divisions in *S. Puerariæ*.

Metaphase.—The spindle is extremely long and slender, and stretches across the full length of the nucleus (fig. 41). The latter becomes slightly elongated in the direction of the spindle, and the nuclear membrane thereupon quickly disappears. The nucleolus is now much reduced in size and lies near the periphery of the nucleus at some distance from the spindle. A few disintegrating chromatin granules may still be seen near the junction of the ground substance of the nucleus and the surrounding cytoplasm. As the nuclear membrane disappears, nucleolus and granules pass out, and become lost to view among the numerous dark granules of the cytoplasm. No swelling of the membrane prior to its disappearance, such as STEVENS reported, occurs in this species. During prophase it is extremely delicate, but, until metaphase is established, it remains sharply differentiated. When the large size of the original nucleolus, and even of the remnant that persists at metaphase, is considered, the chromosomes are unexpectedly minute. Their form is almost spherical, and they lie close to one another in the equatorial region of the slender pointed spindle. The cytoplasm, on the disappearance of the nuclear membrane, has been stated by previous writers to "encroach upon" the nuclear area. The sharpness of the distinction between the two regions in the present species is admittedly gradually lost, but, even at the completion of the second mitosis, the limits of the primary nucleus may still be recognised. The eventual uniformity in the structure of the protoplasm seems to be secured rather by an opening up of the portion which is of nuclear origin by progressive vacuolisation working from the periphery inwards than by an actual advance of the cytoplasmic region into the area previously occupied by the nucleus.

Anaphase.—As the daughter chromosomes separate and pass to the poles, the individual members of either group maintain their relative distance apart for almost the whole length of the spindle, and only as they approach the poles do they exhibit a tendency to come together. In the region of the spindle lying between the separating groups the fibres remain parallel until the chromosomes have nearly reached the poles, when elongation of the spindle and a narrowing of the central region begin. The spindle, to begin with, is long, and it is customary in the primary division for only a slight further elongation to take place, so that sometimes the daughter nuclei are formed within the original nuclear plasm. The latter follows the spindle in such elongation as it undergoes, and constitutes the "halo" described for other species. No trace of centrosomes or polar radiations could be detected.

The telophase of the primary mitosis was not found.

The Division of the Secondary Nuclei.

Resting Stage.—The secondary nuclei, even those which result from the first few divisions, are unexpectedly small. The nucleolus remains conspicuous in the successive generations of nuclei until the sporangia approach zoospore formation, by which time the nuclei are extremely minute, and contain only two or three chromatin granules, so similar in size that a distinct nucleolus can no longer be recognised (figs. 42, 62). The linin in the nucleus occurs as slender strands, and, until prophase commences, the nuclear vacuole is comparatively free from the anastomosing threads, such as those which constitute the reticulum in the maturing primary nucleus. The nucleolus, in addition, is excentric in position in the secondary nuclei. In the case of *S. Taraxaci*, Bally (1) considered it improbable that the chromatin granules of the secondary nuclei were derived from the nucleolus in the same way as those of the primary nucleus. Their size, which is large when compared with that of the nucleolus, and the absence of a linin reticulum, which, being a product of the nucleolar discharge would confirm the previous occurrence of a nucleolar discharge, seem to militate against the possibility of a parallel method of derivation of the granules. However, the frequent occurrence of chromatic globules upon the surface of the nucleolus so closely resembles the accumulation of chromatin granules on the nucleolus of the primary nucleus that the origin of at least a certain proportion of the chromatin granules of the secondary nuclei may safely be said to be nucleolar. As it is probable that the method of production is uniform for all the granules, it has been concluded that in *S. endobioticum* at least, the chromatin granules, as a whole, probably owe their origin to some form of nucleolar discharge.

Prophase.—At the beginning of prophase, the chromatin granules on the nuclear membrane stain less deeply and diminish in size, and, as they undergo solution, a linin reticulum makes its appearance across the vacuole of the nucleus (fig. 43). The nucleolus buds freely and the granules formed travel to the periphery, and in turn they, too, fade and disappear (fig. 44). During this process the final density of

the linin reticulum is attained, and the vacuole, as in the prophase of the primary division, thereby becomes filled with a homogeneous substance. As in the latter also, the fine linin strands, which will enter into the constitution of the spindle, may be seen at this stage attached to the nucleolus, and extending from it in sweeping curves to the nuclear membrane (fig. 45). When the nucleus has become almost free from granules, a globule is given off from the nucleolus, and at times it is almost as large as the remainder of the nucleolus (figs. 46, *a*, *b*, *c*). The chromatin of this globule becomes concentrated in the free end as it separates from the nucleolus, and it is believed that it is from this that the five chromosomes are formed (fig. 47). When the difference in size between the primary and the secondary nuclei is considered, a corresponding inequality might be expected in the size of their respective chromosomes. It is not more pronounced, however, than is that between the chromosomes of secondary nuclei of different generations.

Metaphase.—The spindle of the secondary nucleus, when compared with that of the primary, is short, relatively broad, and ends abruptly in a sharp point immediately within the nuclear membrane (fig. 48). The nucleolus at metaphase may be seen as a distinct granule lying between spindle and membrane, and it constitutes the only free chromatin within the nucleus. Upon the disappearance of the membrane, slight elongation of the spindle sets in before the chromosomes divide, and the nucleolus at the same time passes to the periphery of the nuclear region (figs. 49, *a*, *b*). Prior to division the chromosomes elongate and the daughter chromosomes then appear as minute spherical granules (fig. 50).

Anaphase.—As in the primary mitosis, each chromosome maintains its distance from the other members of its group during the passage to the poles (figs. 51, *a*, *b*). The final elongation of the spindle varies, but on the whole it is not as great as that found by BALLY (1) in *S. Taraxaci*. Where it is slight it is often accompanied by an extension of the nuclear plasm, but as the chromosomes approach one another near the poles they pass beyond the limit of this plasm (fig. 51 *b*).

At *telophase* the chromosomes unite to form a darkly staining globule, in which the end of the spindle seems to be included (figs. 52, *a*, *b*). The spindle, which until late anaphase was maintained at the full width of the equatorial region at metaphase, is drawn out at telophase into a thin, dark, curved strand, connecting the daughter nuclei. A vacuole, which quickly enlarges to a conspicuous size, then appears round the chromosomes as they unite to form the nucleolus (fig. 53). A few linin threads make their appearance, and the nucleolus, after reaching a certain size, begins to give off portions of its substance as chromatin globules. Several globules are usually extruded at one time, and each nucleus as it matures normally undergoes two or three periods of nucleolar budding in rapid succession, until eventually the resting stage, with its numerous chromatin granules, is attained. In figs. 54 and 55 are depicted almost parallel stages in the evolution of the resting condition of large and of small nuclei respectively. The general method of development is the same

whatever the size of the nucleus. Different stages in the budding of the nucleolus for the formation of the granules are to be seen in fig. 54 *b*, and figs. 55, *b*, *c*. No trace of centrosomes or asters was found, but since in the two species in which their occurrence has been reported they are present during only a short portion of telophase, it is possible that suitable stages have not yet been obtained in *S. endobioticum*.

Before the segmentation of the prosorus into sporangia the nuclei after each division regain almost their original size before again dividing, but after segmentation has taken place there is a noticeable difference in the size of the nuclei of succeeding generations, and when a few further divisions have occurred they are reduced to the minute nuclei of the zoospores.

The extra-nuclear chromatic granules which from an early stage are conspicuous in the cytoplasm of the parasite begin to disappear as nuclear division sets in. They are still quite distinct, however, at the time of segmentation, and only when the sporangia are nearing the formation of the zoospores do they completely vanish (fig. 62). Both PERCIVAL (35) and BALLY (1) describe a frequent occurrence of nuclei of unequal size in the prosorus of *S. endobioticum*. In spite of an exhaustive search, their results could not be confirmed, for no unequal nuclei were observed in the prosorus. In the sorus, however, one sporangium was seen which was mature and just forming its zoospores, and which possessed a large nucleus, differing widely in size from the three hundred other normal nuclei. But this was a case of arrested development and not of unequal rapidity of division. Moreover, its occurrence was unique. BALLY (1) suggests that the inequality of what he believes to be nuclei is possibly to be referred to amitotic division. Any method of division other than mitosis, however, certainly does not occur in either prosorus or sorus. In the illustrations given by these writers in which large nuclei and somewhat smaller nuclei are shown, the apparent difference in size would seem to be explained by differences in the angle at which nuclei of equal size were cut. Where the difference in size is very much greater, however, it seems probable that the chromatic granules of the cytoplasm have been mistaken for nuclei. This suggestion is supported by the large size of the normal nuclei in each figure, for at the stage at which such nuclei are present in the parasite granules are always numerous in the cytoplasm. From the present work it has been concluded that mitosis is the sole method of nuclear division in both prosorus and sorus, that inequality in the size of the nuclei is extremely rare, and that when it does occur it is probably abnormal.

The Segmentation of the Prosorus.

There are at present two theories concerning the method of segmentation in the genus. The earlier, which was proposed by HARPER (15), and has since been widely accepted, supposes that a shrinkage of the protoplasm of the prosorus occurs, that water is given off, and that invagination of the membrane of the body at several

points on the surface then follows. The channels of separation thus formed progress inwards, and finally meet at the centre, thereby cutting the body into multinucleate segments. These segments may be further subdivided into uninucleate "protospores," or develop from their multinucleate condition without subdivision, according to the species. Until KUSANO'S paper appeared, this theory was largely held, and KUSANO (22) himself believes that some prosori divide by a somewhat similar process. Nevertheless, for other prosori, he describes a method of segmentation in which the preliminary shrinkage is omitted, while, in place of the cleavage channels of the invagination theory, cleavage membranes are formed between the nuclei, although independently of their division.

In *S. endobioticum*, the prosorus, at the time of cleavage, contains about thirty-two nuclei, and, as they are distributed evenly through the cytoplasm, the number included in each segment varies according to its size. The walls appear simultaneously throughout the organism. Their formation takes place independently of nuclear division, although it often begins when the nuclei are in early telophase, in which case it is completed before they again divide. There are usually not fewer than four sporangia in a sorus, while there may be as many as nine, but five and seven are the numbers most frequently found (fig. 60). In two exceptional cases, division was omitted altogether, the prosorus giving rise directly to zoospores.

The first indication of the approach of segmentation is given by the protoplasm becoming slightly denser, and the size of its vacuoles diminishing, along the course of the future walls (fig. 56). In the middle of these areas, an especially deeply-staining layer is differentiated, which, before it is sharply delimited, follows a minutely undulating path as it skirts the vacuoles (fig. 57). The layer then shrinks in width and becomes less undulating, while in the middle of it there is differentiated a delicate, clearly defined membrane (fig. 58). As the latter becomes firmer, the vacuoles in the adjacent protoplasm gradually regain their normal size. When fully formed, the walls intersect one another at wide but definite angles (fig. 59). During the last few years, somewhat undue stress has been placed upon the shape of the sporangia when enclosed in the sorus—whether their surfaces are sharply angled and fit together, or rounded and merely adjacent—but both these relations are exhibited by all sporangia in the normal course of development. When young they fit together, as the membrane of separation between any two was originally common to both, but, as they enlarge, they naturally become rounded, although mutual pressure may keep the surfaces in contact still flat. The shape of the sporangium is thus largely dependent upon the accident of spatial limitations, and it should not be regarded as a character of diagnostic value, as it has been in several instances.

The Sorus.

General Development of the Sporangia.

After the formation of the sporangial walls, the nuclei divide repeatedly, and decrease in size at each division, until the two to three hundred minute nuclei of the zoospores are formed (figs. 60–62). Mitosis takes place simultaneously throughout each sporangium, but is independent of the process in the other sporangia of the sorus. The cleavage walls, as a rule, are formed during the telophase of the prosoral nuclei, and this phase is usually completed simultaneously in all the sporangia. Intersporangial variation is begun owing to the different durations of the ensuing resting period. A difference of a complete generation may be introduced at the next division through the nuclei of only a proportion of the sporangia participating in it (fig. 60). But gradual variation, resulting at maturation of the sorus in a more or less regular series of nuclear stages, is, on the whole, more common. The final and maximum difference exhibited by the sporangia of any sorus has not been found to exceed that of two nuclear generations, and usually it is considerably less. In one mature sorus observed, five out of the total seven sporangia were at exactly the same state of development, but this was an exceptional case. Fig. 61 shows a sorus with three sporangia whose nuclei are at the same stage.

The substance of the extra-nuclear chromatin granules decreases in amount during the further development of the enlarging sporangium, and finally they disappear. At the same time, the protoplasmic vacuoles, which are conspicuous during the prosoral and early soral stages, become much reduced in size. A portion of a sporangium, shortly before the delimitation of the zoospores, is shown in fig. 62. The nuclei are numerous and small. Their chromatin granules are almost equal in size, and lie upon the nuclear membrane, while joining them here and there are short linin strands. No granules are to be detected in the protoplasm. Occasionally, the sporangia are liberated at this stage, and the formation of the zoospores then takes place as they lie free upon the surface of the tumour, or, if the sporangia have not been completely wetted, as they float upon the water which was the cause of the rupture of the host cell. But usually before the sporangia are liberated, the zoospores are at least delimited even if they are not completely separated.

The Formation of the Zoospores.

Before the formation of the zoospores begins water must be absorbed in considerable quantities by the sporangium. The general appearance of the protoplasm then becomes lighter and on careful examination it is evident that the somewhat transparent effect is the result of slight vacuolation of the protoplasm in a small region immediately surrounding each nucleus. Separating the vacuolated areas, and intersecting one another roughly at right angles, are short strands of protoplasm which

have retained their original density, and by the combined intersections of which the delimitation of the zoospore areas is effected (fig. 63). In each area, lying at the edge of one of the delimiting strands a single chromatin granule can be detected, and in well stained preparations a dark but delicate thread is also visible connecting it to the nucleus (fig. 63). This granule is the blepharoplast, and both it and its connecting thread can be readily detected in the zoospore in all subsequent stages until fusion or entry into the host plant takes place. Of the origin of the blepharoplast little can be said. It is usually held that it is to be traced to structures present in the spindle of the preceding mitosis. In the present case, however, nuclei at the resting stage have been seen which had not formed blepharoplasts and which yet were apparently of the same size as those of the zoospores; but it is difficult to secure thoroughly satisfactorily fixation of the sporangium at this stage and the slightest contraction is immediately reflected in a reduction in the size of the nucleus. The smallness of the nuclei in the last few generations moreover, renders somewhat insecure the assumption that any particular nucleus belongs to the last generation.

As the sporangium continues to absorb water minute vacuoles make their appearance in the centre of the delimiting strands. They gradually increase in size and the young zoospores are forced slightly apart, although for some time each still remains connected to its neighbours by fine threads of protoplasm (fig. 64). As separation continues variation occurs in the length of these connecting threads (fig. 65). Attached to each zoospore is one thread which becomes much longer than the others and continues to increase in length after the shorter ones disappear. The persistent thread is attached at one of its ends to a blepharoplast, and at the other to another zoospore (though not to its blepharoplast), or as more often happens to other similar long threads (fig. 66). As the full length is attained the threads break apart at their junction, or from the second zoospore as the case may be, and each zoospore, equipped with a single long cilium thereupon becomes free in the sporangium. No case of the direct attachment of a cilium to two blepharoplasts has been found.

The Liberation of the Sporangia.

About the time of the cleavage of the prosorus into sporangia the cells of the tissue lying beneath the host cell undergo several divisions in which the newly formed walls lie close together and parallel to the surface of the tumour, presenting an appearance not unlike that of a cambium. During the maturation of the sporangia these cells enlarge in the upward direction, and in doing so push the host cell and the rosette above the level of the surrounding surface, the epidermal cells adjoining the rosette meanwhile dividing to keep pace with the expansion (fig. 71). As the sporangia swell at maturity they exert great pressure on the host cell from within, and at the same time pressure is exerted on the host cell from without by the expanding cells of the tissue below. As the result of the two pressures the host cell bursts at the small exposed region of the outer wall between the cells of the

rosette and the sporangia, if they are not jerked out, at least lie exposed to the exterior. The enlarging cells of the host tissue below sometimes grow to an enormous size, even greater than that of the cells of the rosette (fig. 67). In *S. puerariæ* they expand still more and raise the liberated sporangia high above the surrounding host tissue, and the scattering of the sporangia of *S. fulgens* upon the surface of the host "wie lose Uredosporen," is probably to be referred to a similar process.

The Formation of the Rosette.

The young prosorus usually reaches its full size before any indication of an upward enlargement can be detected in the ring of epidermal cells in contact with the host cell. Elongation begins, however, shortly before the contents of the prosorus migrate. It may be confined to the single ring of cells in contact with the host cell, or those adjoining these may also participate, although to a smaller extent. During the enlargement of the prosorus, and later of the sorus, the rosette cells keep pace with the expansion of the host cell, but a small central area on the epidermis of the latter always remains free. The mature rosette exhibits wide variation in size and structure. The longer cells are usually divided by transverse septa, but when the rosette remains small, division may be omitted. They are remarkable for the extraordinarily rapid growth which can be induced by raising the temperature. When removed from a temperature of 16° C. and placed in an incubater at 20° C. they will elongate to twice their original length within four hours. These cells have a shining refractive appearance in the fresh state, and the cell sap appears to contain oil since it darkens with osmic acid; the blackening, however, is never so deep as that of the vacuoles in the resting sporangium or prosorus. (For stages in the development of the rosette see figs. 68–71.)

The Discharge and Structure of the Zoospores.

On the separation of the zoospores the sporangium, if covered with water, becomes clear and almost transparent. As it continues to enlarge, a hyaline projection, sometimes followed by a second or even a third, appears at one or more of the angles on the surface (fig. 72). Rupture will take place at one of these projections. If several are present, it will be at the last formed; but if only one develops, it may take place through it or through an unswollen region of the membrane. Vigorous movement of the zoospores within the sporangium has not been found to occur before at least a few zoospores have been discharged. The hole in the sporangial membrane is really a small slit. The behaviour of the zoospores at discharge depends upon their age, that is, upon the time that has elapsed since they were completely separated from one another in the sporangium. It frequently happens that sufficient water is present to secure the formation of the zoospores, but not to effect the liberation of the sporangia from the host cell. Zoospores, when fully formed, can remain unharmed in the sporangia for nearly a week, provided the sorus is not ruptured.

On the addition of water after such a period of delayed development, the zoospores are usually liberated from the sporangium simultaneously with the discharge of the latter from the host cell. When the bursting takes place suddenly in this way, the opening in the sporangium may be a slit of considerable size. The zoospores dash through it in a condition of violent activity, and it is difficult to follow the movement of any zoospore for more than a few seconds. When a few zoospores have escaped, those inside become as active as those without, and in time they too dart through the opening, and the sporangium eventually is left in a collapsed condition, with perhaps one or two zoospores caught in its folds. The most active movement, which consists of a dashing to and fro over short paths, may last from 10 to 20 minutes. Then the rate is diminished and the characteristic jerk of the cilium and body at each change in the direction of the path can be seen. In from 30 to 40 minutes movement will gradually cease and the zoospore when not on the surface of an object suitable for penetration suffers disintegration. The surface of the host tissue seems to offer an attraction to the zoospores. Again and again, when in their most active condition, they dart straight to the epidermis but fly off again immediately. As their speed is reduced, however, they may be observed to pause for a moment before returning to the open a little more slowly than they advanced. Each time they return to the host surface they stay longer, and each time they reduce the length of the backward path until finally quite 90 per cent. remain upon the plant tissue. Certain of the zoospores may swim away, but from the relations between host cells in which sori had developed and zoospores at rest on the surface, in material in which discharge of the zoospores took place under natural conditions, it has been concluded that the majority of the zoospores do not travel far from the host cell in which they develop.

On the other hand, when immature sporangia are liberated from the host cell a certain time necessarily elapses before the zoospores are discharged, the length of the period varying according to the initial state of development of the zoospores. When these sporangia rupture the hole is usually small and the zoospores slip out one at a time and remain inactive just outside the aperture. Those inside may then become active, but their movement is not so rapid as that of perfectly mature zoospores. Frequently it is only slight, and they seem to drift out rather than actively to seek the open, and they then may remain congregated in a mass outside the opening for five to ten minutes before movement begins. Sometimes, indeed, they do not become motile at all, but simply die where they lie. Usually, however, a sluggish movement sets in and they go through the same activities as the mature zoospore, although at a slower rate. Eventually they, too, come to rest upon the epidermis of the tumour, or of new potato tissue if it happens to be close at hand. The probable cause of their congregating outside the opening of the sporangium is that the cilia have not yet become free, and the zoospores in consequence are still joined together.

The activity of mature zoospores is maintained under wide variation in the external conditions. It will continue in light and in darkness, although a very bright light prevents the speed from reaching its maximum. The temperature may range from 12° C. to about 19° C. without inducing any inhibitory effect. If it is raised above 20° C., however, movement is checked. The media used for the discharge of zoospores, named in order of suitability, were rain water, conductivity water (distilled over glass), and tap water.

The zoospore, which is about 1·5 μ long, is oval in shape, but tapers slightly at its anterior end where there is a large clear refractive spot (fig. 73). The main portion of the body is slightly more dense in appearance. The cilium, which is of the characteristic chytridian length, is attached to the broader posterior end, and provided the movement is sufficiently slow can be observed in the living zoospore.

At first, difficulty was experienced in retaining zoospores upon the slide for the purpose of fixation, but eventually a simple method was evolved by which few zoospores were lost during manipulation, whether they occurred singly or were undergoing the process of fusion. The adhesive consists of equal parts of albumen and pure glycerin, and is made up each time before use. It is spread as thinly as possible by means of a second slide and not by hand, for by the latter method irregularity is introduced in the evenness of the film. It is then wiped off except for a small area in the centre of the slide about half an inch in diameter, and upon this alone the zoospores are placed. The zoospores meanwhile have been prepared in water upon a second slide, and when they are at the stage at which fixation is desired they are drawn up into a graduated tube of which the bore of the free end is no wider than a hair. To the wider end of the tube is attached about three-quarters of a yard of flexible rubber tubing and this in turn is connected to a mouthpiece, by the use of which greater freedom is left to the hands. The process of picking up the zoospores is carried out under the microscope, and they are then deposited on the prepared area of adhesive. If the number of zoospores in the first drop is insufficient, more drops may be added until a practicable number is obtained. Finally, the drop is left to evaporate until the slide can be handled without danger of spilling the drop. It is then placed for a few moments in a Roux tube containing crystals of iodine. The lower end of the tube is slightly warmed and the zoospores are killed and temporarily fixed by the fumes; fumes of osmic acid were sometimes used in place of iodine. The slide is then kept in a desiccator until the drop has evaporated, when it is placed in vapour of alcohol. On the coagulation of the film of albumen it is brought down through changes of alcohol to water, and a little FLEMMING'S fixative (the strong solution diluted with an equal quantity of water) is placed upon the area and left there for two hours. The zoospores are now permanently fixed. Henceforth the slide may be treated as in the case of an ordinary mount, and the objects may be bleached and stained at will. For the staining of the nucleus BREINL'S triple stain gives excellent results, but for that of the blepharoplast, connecting

thread, and cilium the best stain is HEIDENHAIN'S iron alum hæmatoxylin. The cilium is more sharply defined when bleaching is omitted.

The nucleus, which is conspicuous, lies in the narrow anterior end of the zoospore (fig. 74*a*), and together with one or two small fat droplets in its neighbourhood is responsible for the appearance of the large refractive spot in the living zoospore (fig. 74*b*). In *S. puerariæ*, on the other hand, KUSANO describes the position of the nucleus as central, while the fat droplets alone lie further forward. In *S. endobioticum* the nucleus is practically spherical and contains a large central vacuole. Three or four chromatin granules of about equal size rest upon the membrane, and in some cases one or two linin threads may be seen extending across the vacuole from granule to granule. Very little protoplasm is present in the zoospore. It is slightly denser in the region immediately beneath the nucleus, and from this part it extends as delicate threads round the periphery of the body, the central region being occupied by a large vacuole, which, however, does not contain oil. The blepharoplast, which is usually a little smaller than the chromatin granules of the nucleus, is to be found at the surface of the zoospore, at the end opposite that occupied by the nucleus (fig. 74*a*). The thread connecting blepharoplast and nucleus frequently lies in a strand of protoplasm, and so follows the contour of the body in a curved path.

Both zoospores and zygotes adhere firmly to the surface of the host plant once they have come to rest upon it, and every stage, from the zoospore immediately after its discharge to the zygote as it penetrates the epidermis, can readily be found on the surface of the tumour.

The Entry of the Soral Zoospore.

The zoospore, when at rest upon the epidermis of the host plant before entry, lies usually upon its side, and the nuclear end is thus raised above the surface (fig. 77); but occasionally the zoospore becomes attached by the anterior end, when it sinks slightly upon the host extending the surface of contact, and the form changes from a rounded to a low oval. The cilium now undergoes contraction. This process begins at the free end which becomes swollen, and the shortening and swelling then extend down the cilium towards the blepharoplast (figs. 75*a*, *b*), the cilium being eventually reduced to a short rod-like body (fig. 75*c*). The region at the point of attachment to the zoospore is the last to remain slender, but the substance of the cilium is eventually drawn back upon it, and a beaded darkly staining structure results (figs. 75*d*, *e*). In the last stage it is reduced to a heavily staining globule, which rests upon the surface of the zoospore near the blepharoplast (fig. 75*f*). Whether this globule is thrown off or withdrawn into the body of the zoospore cannot be definitely stated, but as many stages of zoospores in the process of penetrating the host have been found it is unlikely that the withdrawal into the zoospore of such a deeply staining body could be overlooked. In all probability, therefore, the cilium is thrown off after being contracted.

The entry of the zoospore into the host may begin soon after the cilium begins

to shorten (fig. 75*c*), but it is more often postponed until the contraction is almost complete. A change in the nucleus is the first sign that the process of entry is beginning for a projection appears on the side of the nucleus towards the epidermal surface. This projection lengthens (fig. 76*b*) and finally reaches the surface of the zoospore, where it lies in contact with the host wall. By this time the projection consists of a chromatic granule on the zoospore surface and a deeply staining thread of nuclear material, connecting the granule to the main body of the nucleus (fig. 77, where the blepharoplast is also visible). In fig. 76*a* the projection may be seen as it leaves the nucleus, while in fig. 76*b* it has advanced almost to the surface.

The earliest stage of actual penetration that has been observed shows the nuclear projection piercing the wall and extending slightly beyond into the cell. The projection, however, is so small that the thin layer of cytoplasm which doubtless covers it cannot be detected (fig. 78).

When the nucleus is passing through the pore the long heavy strand into which it is extended can often be traced from the exterior to the interior half of the zoospore without a break (fig. 79). In fig. 80 the first part of the projection has passed within the wall, and in fig. 81, which is another surface view, a small portion of the body of the zoospore has also entered. If the nucleus lies close to the surface of contact, great elongation will not occur as it approaches the pore, but usually it lies at a little distance, in which case it is drawn out into a narrow strand extending at times half-way across the zoospore (fig. 82). In fig. 83 the nucleus has almost completed its entry, while in figs. 84 and 85 it is wholly within the host cell wall, while only a vesicle of protoplasm remains outside. The last stage of entry is shown in fig. 86, where both cytoplasm and nucleus have completely entered, but the latter still remains connected to the pore, although its major portion has already resumed its spherical form. The posterior nuclear extension is soon drawn to the nucleus proper, which then becomes completely spherical. The central nuclear region does not open up to form a vacuole similar to that of the zoospore nucleus, but the granules at the periphery lie close together and merge into one another, so that the nucleus as a whole gives a deep staining reaction.

The parasite on entry represents the youngest stage of the prosorus. It soon moves from the epidermal wall of the host cell. Sometimes its route lies close against the side wall of the latter (figs. 87, 88), or it may take a more diagonal course until it approaches the host nucleus. The latter is large, and may extend almost across the cell, but there is usually sufficient space for the small parasite to pass beyond it. In the normal rapidly dividing epidermal cell, which is long and narrow, the organism passes directly to the inner end before enlarging to any extent. But in other cases enlargement may commence before it moves far from the epidermal wall, in which event it does not penetrate deeper than the neighbourhood of the nucleus (fig. 90). The protoplasm of the organism increases rapidly in amount, and is broken up into a uniform network by the formation of numerous small vacuoles.

Enlargement of the nucleus meanwhile keeps pace with that of the cytoplasm. The production of chromatin has taken place so rapidly that it now stains in a more or less homogeneous manner, and closely resembles the nucleolus of the more mature prosorus (fig. 89). At the same time a small nuclear vacuole is formed round it this quickly enlarges to the dimension characteristic of it in later stages (fig. 90) and on the appearance of a few linin strands within it the typical nucleus of the young prosors is evolved.

The outline of the history of one complete soral generation has now been traced It remains to follow the more normal course of events in which fusion of the zoospores takes place after the formation of the first generation of sori. For the production of several soral generations is rather a repetition of a particular phase owing to the subjection of the organism to certain conditions at a critical period, than the normal sequence of events in the life cycle.

The Fusion of the Soral Zoospores (Gametes).

The fusing cells are exactly similar, so the process is one of isogamy. Those zoospores whose movement is sluggish from the time of their discharge have not been found to fuse. The fusion of active zoospores, however, may be watched beneath the microscope. No attempt at fusion is made when movement is at its height, but by the time the perceptible pause has been introduced at the change in the direction of the path it may be observed that two or more zoospores occasionally dart in the same direction for a few seconds. As the pace is further reduced this develops into a deliberate following of one zoospore by a second. On one occasion two zoospores from different sporangia approached each other at a steady pace. They met, then one turned and swam slowly away at an angle to its original direction. The second swam after the first at an equal pace, following immediately the five or six alterations in the path of the leading zoospore which were observed during about half a minute. Then the second zoospore stopped, whereupon the leader turned and came back, and after gliding along the stationary zoospore once or twice settled beside it, and in time the two fused. Frequently zoospores meet, remain together for a moment, and then separate rapidly without exhibiting any further tendency to fuse. Fusion is usually effected between a pair of zoospores, one of which has already come to rest before the second apparently by accident slowly swims up, touches it and, as if as the result of contact, loses its activity. It may, however, be seen to move along the surface of the stationary zoospore before finally coming to rest. When fresh zoospores join a stationary group the pairs are frequently rearranged, and some of the original members may swim away.

The Conditions Necessary for Fusion.

It is believed that fusion does not occur between individuals originating from the same sporangium, for whenever isolated ruptured sporangia were found on the surface

of the tumour only zoospores were to be seen in their neighbourhood; whereas when the remains of several ruptured sporangia lay near one another, zygotes were present in their vicinity. Fusion, however, can take place between zoospores originating from sporangia of the same sorus, for zygotes are frequently to be seen at the base of ruptured host cells when no other sori lie near; but as the sporangia are usually at different stages of development when liberated from the host cell, the zoospores will probably not be discharged at one time, in consequence of which the fusion of such nearly related gametes is possibly not of frequent occurrence under natural conditions. It is not usual for more than five nuclear divisions to occur between the segmentation of the prosorus and the formation of the zoospores. In the event of the fusion of gametes from the same sorus the nuclei will therefore probably have developed independently for not more than five generations. It is possible that independent nuclear development may have originated further back in the multinucleate prosoral stage but of that we can have no proof.

One exceptional case observed is worthy of mention. A large sporangium in an unruptured sorus contained from 500 to 600 zoospores. The tissue in the neighbourhood was decaying, and the cells adjoining the host cell were already dead. In addition to the zoospores, the sporangium contained 18 zygotes. At first, it seemed probable that the last nuclear division had been omitted in their case, and that they were simply zoospores of twice the normal size. But in three cases the nuclei were double structures—which, however, were not the daughter nuclei of a delayed final mitosis. They were, in fact, ordinary zoospore nuclei in the process of fusion. The sporangium was unusually large, and it may be that some accident prevented the cleavage walls from forming. That, however, does not alter the fact that intra-sporangial fusion of gametes took place in these few instances, an occurrence which serves to strengthen the belief in the normality of fusion between gametes of the same sorus. The wide question arises as to whether, in the particular case mentioned, the organism under the stimulating effect of the presence of toxic substances may not have had recourse to an earlier (? more primitive) method of sexual reproduction.

It has often been stated that, in nature, sori are developed in spring, while resting sporangia do not appear until late summer and autumn, by which time sori are no longer to be found, and it is hinted that the difference in the temperature of the seasons is responsible for the formation of one or other of the types of reproductive bodies. That more sori are formed at the beginning of the season and more resting sporangia at the end is certainly true, but resting sporangia may and do occur quite early, and sori similarly may be found quite late. Temperature is not the decisive factor, for the formation of resting sporangia may be induced at 18° C., while series of sori may be continued at 12° C. What is really essential for the fusion of gametes and the formation of resting sporangia appears to be that in two sporangia a maturation period should intervene between the formation of the zoospores and their discharge from the sporangium, and that the discharge from both

sporangia should be practically simultaneous. In spring and early summer, when there is an abundance of water in the soil, it is probable that one or other of the conditions will not be fulfilled, so zoospores remain unfused, and give rise to more sori. If, however, water is lacking at the critical period the zoospores have time to mature, and, the longer water is absent, the more sori will there be with their contents in the required condition. At the advent of water, therefore, numerous mature sporangia will be liberated together, and immediately discharge their zoospores (gametes). After the normal period of activity the latter will fuse, and resting sporangia will ensue.

The belief in the necessity of a maturation period intervening between gamete formation and discharge, before fusion can occur, is the outcome of an accident. A tumour bearing mature sori was left unwatered; some days afterwards, pieces of the surface of the tumour were placed in a drop of water and examined. The zoospores were seen to be extremely active, and, when their speed was reduced, to fuse in pairs. Water was then deliberately withheld from two other tumours bearing sori in a similar stage of development; on the fourth day the sori were examined in water, and in both cases the gametes fused. Sori and resting sporangia can be distinguished with a lens when in the living tumour, so the experiment was repeated on a larger scale without removing the sori, in order that the sporangia and gametes might remain under natural conditions. Tumours were chosen in which only maturing sori, and no resting sporangia, were present; they were well watered, and then left for four days, when they were again watered. In a few days, young resting sporangia were to be seen. On the other hand, similar tumours, which had been watered daily to serve as controls, still bore only sori. The experiment has been repeated many times, and in not one case have the matured gametes failed to fuse and give rise to resting sporangia, or the immature zoospores to give rise to sori. Moreover, the formation of sori, as of resting sporangia, has been induced at will over a wide range of temperature.

The Cytology of the Fusion Process.

When the gametes are fusing, they lie with their longer axes parallel or slightly inclined, and with their anterior ends facing usually in the same direction. In fig. 91*a*, two gametes may be seen approaching one another, while in fig. 91*b* they have touched. In fig. 92 they are pressed together, but the membrane of each is still distinct. The membranes, however, soon disappear at the surface of contact, and only a slightly denser strand of protoplasm then marks the line of union (fig. 93). The nuclei now approach each other, and eventually meet in the region of cell fusion. Stages in the approach of nuclei are shown in fig. 94 *a–c*. After meeting, the nuclei become pressed together, and the surfaces in contact are flattened (figs. 95*a*, *b*, *c*). The line of junction shows as a broad dark strand as the result of the accumulation of peripherally arranged chromatin of the two

gamete nuclei. The chromatin from the area of junction then moves to the poles of the nuclei, leaving the joined membranes clear (figs. 96*a*, *b*). The polar accumulations, however, soon disappear, the chromatin becoming distributed regularly on the outer surfaces of the nuclei (fig. 97). The polar depressions resulting from the junction of two oval bodies can no longer be detected, and the membrane between the nuclei ceases to be visible. This spherical fusion nucleus may contract slightly at its formation, and its chromatin forms a dense peripheral layer (fig. 98). This stage quickly passes, and the nucleus enlarges again, while the chromatin at the same time becomes resolved into granules (fig. 99), which, when the nucleus has reached its full size, are seen to be connected by an intricate network of linin threads (figs. 100, 101*a*, *b*).

Normally, the nucleus of the zygote is in this open, resting condition before infection occurs, the whole process of cell and nuclear fusion having been accomplished on or near the surface of the potato tissue. Certain abnormalities have been observed. They usually take the form of what is apparently the late arrival of a second zoospore after the first has begun to penetrate the epidermis. Provided penetration has not advanced too far, fusion is effected, and the zygote nucleus is formed. The fact that two zoospores have already fused does not act as a deterrent to a third, and cases have been found in which a zoospore is firmly perched on the top of an entering zygote.

No real membrane is formed round the zygote, but a slight condensation of protoplasm takes place at the surface, and, should plasmolysis occur, this outer layer remains in position, while the protoplasm within contracts (fig. 107).

Fig. 102 shows an aggregation of zygotes in a depression on the surface of a tumour. Two ruptured sori lie opposite the cavity at a little distance from it, but they are not shown in the figure. With the exception of a few zoospores which had settled in another depression they all entered the cavity depicted and there fused. No zoospores or zygotes were to be seen on the level portion of the tumour where cell division was not taking place rapidly, yet the cavity had a comparatively narrow opening and lay at a little distance from the sori. This attraction towards regions in which cell division is in progress may be observed all over the tumour.

Different stages of fusion are shown in the gametes and zygotes of fig. 103. In this case they have entered a channel between three rosette cells and there begun to fuse. On the lower cell there are four mature zygotes and several gametes, while above are to be seen different stages in the approach of the nuclei. The number of resting sporangia which occur close together in heavily infected tumours has led some to think that division of the organism after entry is necessary to explain the presence of so many. A glance at fig. 104, however, which is drawn from a section only 4 μ in thickness, shows how unnecessary is such an assumption, for which moreover, we have no evidence. Upon the large crumpled rosette cell at the upper right hand side there were 23 zygotes in addition to gametes. In living material on one occasion

35 zygotes, and on another occasion 26 were seen resting upon one rosette cell. These are admittedly exceptional numbers, but they occurred under natural conditions, and there is no reason to suppose that only a small proportion would have effected entry. In the section figured (fig. 104) one zygote is already completely within the host cell, while three are in the process of penetration, and several others are preparing to enter.

The Zygote.

The Entry of the Zygote.

The process of entry in all three types of penetrating bodies—soral zoospore, zygote, and zoospore from the resting sporangium—is essentially the same. The greater size of the last two bodies affords an opportunity for the observation of a more complete series of stages, but in the case of the zygote the advantage is outweighed by the possibility of confusion owing to the presence of two nuclei, two blepharoplasts and two strands connecting the blepharoplasts to the nuclei. As in the soral zoospore the cilia of the zygote are contracted before entry takes place. The process usually sets in early, before the fusion of the nuclei is completed. Owing to the difficulty in retaining cilia during manipulation it is unusual to find a zygote in which both are preserved, but in fig. 105*a* is depicted a zygote both of whose cilia are undergoing contraction. The process is not proceeding evenly. The shorter cilium is smooth in outline and comparatively broad, but in figs. 105*b*, *c*, two cases are shown in which the reduction has taken the form of a contraction in segments resulting in a series of small heavy joints. The latter type of contraction is the more general. In fig. 105*e* is a zygote just beginning to enter the host plant in which the relation of the various parts may be regarded as typical. The fusion nucleus has attained its final form, and consists of numerous peripheral chromatin granules and a central vacuole. The blepharoplasts and their connecting threads are distinct, and attached to one of the former is a contracting cilium. Between the blepharoplasts on the periphery lies the deeply staining tip of the nuclear projection. Subsequent stages in the contraction of the cilium are shown in figs. 105*f* and 105*g*, but of the fate of the knob after it reaches the surface of the zygote nothing could be discovered. When entry is in progress it is no longer to be seen although blepharoplasts are still visible.

The projection of the nucleus is formed without causing general derangement in its structure. In figs. 106*a*, *b*, *c*, are three stages in the development of the projection as it grows out from the nucleus towards the surface of the zygote in contact with the substratum. After the surface is reached the accumulation of chromatin in the apex of the projection increases in amount and eventually appears as a deeply staining granule, rounded towards the interior and flattened towards the surface of the zygote (fig. 107). One has the impression that the apical granule is being forced against the surface of the host cell which the organism is about to penetrate. This is an interesting example of the "control" by the nucleus of a special cell activity. The

apical granule of the nuclear projection now passes through the host cell wall and stages have been observed in which it is half inside the cell and half out (fig. 108). In fig. 109 the apical granule has passed completely through the wall. Owing to the obliquity of view the connection with the nucleus outside the cell cannot be completely demonstrated. Only nuclear substance can be recognised in the portion of the zygote which has entered and the amount of cytoplasm accompanying the granule seems to be extremely small. In fig. 110 is shown a slightly later stage in which a little protoplasm is already evident round the granule and that portion of the connecting strand which is inside the host cell. In fig. 111 the portion of the nucleus which has entered has begun to expand and has become irregular in shape; in this figure the blepharoplasts are also visible. When the nucleus before entry lies close to the lower surface of the zygote the length of the connecting thread is proportionally reduced, and in fig. 112 in which the nucleus is no doubt about to enter there is no projection, but the nucleus already lies in contact with the host cell. Further stages in the entry of the nucleus and cytoplasm are shown in figs. 113*a* and 113*b*; in the latter the nucleus is half way through the pore.

As the nucleus emerges into the host cell it expands into a dark structure of irregular shape which bears little resemblance to the original nucleus (figs, 114, *a*, *b*). Later, however, the nucleus becomes a more or less spherical body, but still remains homogeneously dark in staining reaction (fig. 115). Eventually the rest of the cytoplasm follows the nucleus through the pore (figs. 116, 117, 118), but as will be seen in fig. 119, the portion last entering appears as a small granule above the pore. After this has passed through the cell wall no trace of the pore can be observed. The nucleus for a short time remains drawn out towards the position of the pore after entry is completed. This extension is soon withdrawn however and the nucleus then becomes a dense homogeneous spherical body lying at the centre of the spherical mass of protoplasm. The parasite now moves down the host cell and begins what may for convenience be considered the first stage in the development of the resting sporangium.*

The Resting Sporangium.

The General Appearance of the Resting Sporangium.

Before entering upon a detailed account of the development of the zygote into the resting sporangium, a rapid glance will be cast at a few typical stages of the young sporangium in the tumour.

The general appearance of the epidermal layer of a tumour bearing resting sporangia in an advanced state of development is shown in fig. 120. The sporangia completely fill the host cells and bear upon their surface a series of ridges, the position

* Percival considers the stage after entry to be vegetative and the reproductive phase to start with the formation of a definite membrane other than the plasmatic one. As, however, the exact time of its appearance is very difficult to determine, it seems more convenient to make the above assumption.

of which corresponds with that of the intersection of the walls of the latter. No sori were present in the region of the tumour from which the section was cut, and the uniform size of the uninfected cells is typical of tumours which bear only maturing resting sporangia. The next six figures are drawn under the same magnification (400) in order to show the increase in size which the sporangium undergoes during its development. In fig. 121 the organism has passed down the host cell as far as the nucleus, but it is too minute to allow the detection of any structure, save the protoplasm and the nucleolus of the nucleus. It has enlarged and passed to the lower side of the host nucleus in fig. 122, and a few chromatin granules can now be seen lying beside the nucleolus in the clear vacuole. Division of the host cell has not yet taken place, but the young sporangium lies in a sufficiently deep position to ensure its inclusion in the lower cell should a transverse wall be formed. In fig. 123, on the other hand, division has taken place, and the sporangium now lies in a sub-epidermal cell. In the uniform mesh of its cytoplasm are several dark chromatin granules, while in the nuclear vacuole a small amount of linin has appeared. The host cell has become much enlarged, as the result of which the protoplasm now extends in long strands across the central vacuole to the parasite. In fig. 124 the deposition of the membrane of the sporangium has begun; the nucleus shows an increase both in its size and in the amount of linin it contains, while in the cytoplasm the vacuoles are becoming numerous. A second division of the host cell is responsible for the forcing of the sporangium of fig. 125 into the third layer of cells. In this example a little starch is present in the tissue of the tumour, but in the host cell the contents are reduced to a thin lining of protoplasm upon the walls and upon the sporangium. The granules in the cytoplasm have now become a conspicuous feature of the organism, while the membrane has developed almost to its full thickness. In the next figure an addition has been made to the membrane of the organism through the deposition of the dead contents of the host cell upon the outer sporangial membrane (fig. 126). In the mature sporangium the membrane thus consists of three layers, of which the two inner alone are of sporangial origin. The innermost, however, is too delicate to be visible under the magnification used. The nucleus in the figure lies in the midst of the enlarging granules of the cytoplasm, but its size is reduced, and under the pressure exerted by the expanding cytoplasm it has become shrunken to an irregular pointed structure. The nucleolus at this stage has given up most of its chromatin and appears pale and inconspicuous against the linin of the vacuole. The sporangium of this figure is at the most advanced stage which is normally to be found in a living tumour.

The Development of the Resting Sporangium.

At the beginning of this stage the nucleus of the parasite is represented as a single chromatic globule lying free in the mass of the cytoplasm (fig. 127). The first step in the development from this to a nucleus of normal type is the appearance of a

clear area round the globule (fig. 128). This area widens, and from the chromatic mass linin threads pass to its external limit, which about this time become sharply marked indicating the existence of a definite membrane (fig. 129). The origin of the clear area is obscure. Whether it arose as a vacuole in the cytoplasm, or was developed as the result of the pushing out of a membrane from the periphery of the chromatic granule, could not be determined.

The period during which the nucleus consists only of a large nucleolus and a clear vacuole is not of long duration, for there soon begins a process of transference of nucleolar substance to the nuclear vacuole similar in nature to that which takes place in the nucleus of the prosorus. As in the latter, the process leads to the depletion of the chromatic substance of the nucleolus, to the appearance of linin in the nuclear vacuole, and to the formation of chromatin granules in the cytoplasm. The transference takes place gradually by a succession of nucleolar discharges which may reach the number of six. This observation does not agree with those of PERCIVAL (35) and BALLY (1), who describe only a single protracted discharge. The process is carried further in the resting sporangium than in the prosorus, for the nucleolus finally yields up all its chromatin and becomes reduced to an achromatic sphere in a shrunken and now probably functionless nucleus (fig. 126).

A stage immediately before the first discharge is shown in fig. 130. The nuclear cavity is now distinct and is traversed by one or two linin strands, while the large nucleolus stains deeply at the periphery. In the next stage small granules may be detected on a limited area of the surface of the nucleolus (fig. 131). They become pushed up above the surface by a slight enlargement of the region beneath them, and at the same time they increase in size until they constitute a compact mass of granules whose united volume occasionally is almost equal to that of the rest of the nucleolus (fig. 132). As the chromatin of the projection becomes dissolved a linin-like substance is revealed between the granules (fig. 133). During the process of the discharge the size of the nucleolus has greatly diminished, and its form may now be oval instead of spherical, but the intensity of its staining reaction at this stage is usually not reduced (figs. 134, 135). The granules of the nucleolar projection just described become completely dissolved and leave only the linin-like material in the vacuole (figs. 134, 135). This is the first addition of nucleolar linin to the vacuole. The irregular linin mass left after the dissolution of the chromatin has been termed by PERCIVAL (35) an "amœboid body." By the time the young sporangium has reached this stage of development, the host cell has probably undergone one division, and thus the first step in the deeper placing of the organism in the host tissue has been taken. Growth and development, however, continue uninterruptedly in the parasite during the host cell division. In the succeeding nucleolar discharges the amount of chromatin given off is greater than in the first, but in other respects they are similar, so a detailed description of the process will not be given in their case.

The large number of the chromatin granules lying in the cytoplasm, which are so characteristic a feature of the mature resting sporangium, appear usually after the second nucleolar discharge has taken place. These granules are formed at first in only a few strictly limited areas which lie symmetrically about the nucleus; fig. 136*a* shows four such areas. They are comparatively large, and their protoplasm stains more deeply than that elsewhere, while upon the strands which traverse them, minute granules can be detected. When the deposition of the granules has once begun, special staining reaction of the areas is no longer evident, but only the strands with their attached granules remain. Before this happens, however, new areas of deeper staining protoplasm appear between the original ones (fig. 136*b*). Several sets of such areas are formed, and the individual areas of each set are smaller than those of the preceding set. Moreover, as they appear and disappear rapidly only a short time elapses before the few large areas of the early series are replaced by numerous small ones of later formation. And these in their turn soon become inconspicuous among the great numbers of chromatin granules produced. But even in the case of granules formed late in the development of the sporangium a grouped arrangement can often be detected, although it may no longer be possible to distinguish any difference in the staining reaction of the protoplasm in their immediate vicinity.

Up to this point the parasite has shown only a plasmatic membrane, but, shortly after the appearance of the chromatin granules in the cytoplasm, the cell becomes bounded by a definite membrane, which at first stains deeply with gentian violet. Microchemical tests were not applied, but there can be no doubt that this new membrane is a definite cell wall.

In the nucleus of the sporangium of fig. 137, what is probably the third nucleolar discharge is beginning, and granules are now developing upon the surface of the nucleolus. A few chromatin granules have been formed in the cytoplasm, and the mesh of the latter, as is usual at this stage, is somewhat dense. In fig. 138 the membrane, although thin, is now quite distinct. In the interval which has elapsed between these two stages, the third discharge—as judged from the size of the organism—has been effected, the chromatin to a large extent has dissolved, and the nucleus has increased markedly in size. It is at about this stage that the nucleus attains its maximum size, when it may be as great as 22 μ in diameter, but, from now onwards, it will gradually decrease. A sporangium at a slightly later stage is shown in fig. 139. The solution of the chromatin in the nuclear vacuole in this sporangium has been completed, and an appreciable amount of linin has been added to the vacuole. The cell wall is becoming denser, and in the cytoplasm there is a marked increase in the number of granules. The sporangium of fig. 140, with its deeply-staining wall and numerous granules, is beginning to present the typical appearance of the sporangia of a tumour during the later half of the season. A slight decrease has already taken place in the size of the nucleus, and this reduction

will continue until it becomes an irregular, slightly staining, and no doubt functionless, structure, lying in the midst of the zoospore primordia. The structure of the sporangium at this stage presents sharp contrasts when stained with FLEMMING'S triple stain. The nucleolus is bright red, the chromatin both of the nuclear vacuole and of the cytoplasm violet, the cytoplasm itself orange, and the membrane deep violet. The staining reaction of the membrane, however, at this stage begins to change. From its first appearance, until it begins to thicken, it stains with chromatin stains, but as it becomes broader and its natural yellow colour is developed such staining is confined to its inner and outer surfaces, and eventually it stains only with such dyes as orange G.

The sporangia in figs. 141, 142, 143, show the early, mid, and late phases of what is probably the fourth nucleolar discharge. The membranes of the three sporangia show a great increase in thickness, and in the cytoplasm the granules are becoming both larger and more numerous as compared with fig. 140. The amount of protoplasm doubtless increases as the sporangia become older, but, during the expansion of the cell, the cytoplasmic vacuoles become so much larger that the protoplasm is reduced to fine strands. In the sporangium of fig. 143 there are two points of especial interest. The protoplasm of the host cell is more dense in the region which lies in contact with the sporangium; this is the first stage in the deposition of the cell contents which results in the formation of the outermost layer of the membrane of the mature sporangium. In addition, most of the granules in the cytoplasm now exhibit a differentiation of structure. The smaller still stain deeply and uniformly with chromatin stains, but in the larger the chromatin is confined to the periphery, while a lighter region becomes visible in the centre (fig. 143*a*). As enlargement of the granules continues, the lighter region is seen to be differentiated into plasmatic material and vacuoles. All the granules enlarge rapidly, and the smaller soon show the same differentiation as the larger (fig. 144). In this figure, it will be seen that several of the larger granules are multiplying by a process of constriction into unequal portions. This process is of frequent occurrence in sporangia of this age, but usually only a small number of the larger granules take part in it. In all the granules—which are actually the primordia of zoospores—a marked increase of chromatin now takes place (fig. 145), and it begins to accumulate at certain points, causing the primordia to become irregular in shape (fig. 146). As the accumulation continues, the chromatin projects from the surface, at first slightly and then more markedly. Finally, the chromatic projections become constricted and severed from the primordia, and they thus lie free in the cytoplasm of the sporangium. From one to five granules are given off at a time by each primordium, and the larger it is the more granules it emits. The process does not begin simultaneously in all the primordia, but eventually it occurs in all, even the smallest. At this stage the presence of the numerous minute granules in the cytoplasm between the primordia lends a peculiar appearance to the

sporangium (fig. 147*a*). The granules, however, soon cease to stain deeply, and usually they have almost disappeared when the primordia are still emitting occasional granules (fig. 147*b*). Soon after they have ceased to be emitted, the only remains of the granules are small, irregular, colourless structures upon the strands of protoplasm between the primordia (fig. 148). Since the longer zoospores emit more granules than the smaller, the inequality in their size is considerably reduced at the close of the process, and they become once more practically spherical. The chromatin is now reduced to a few small isolated granules, lying in the plasmatic material, composing the main mass of the zoospore body. The zoospores have been increasing in size for some time, and the protoplasm, which is now very dense, shows only small vacuoles. Eventually, the few small chromatin granules become replaced by a single larger one, though by what process is not clear (fig. 149).

Before the process of chromatin budding takes place in the young primordia, however, there are usually to be seen in each sporangium a number of exceptionally large primordia. These become constricted into two either during or just after the process of budding. In fig. 150*a* the difference in size of a normal and a large primordium may be seen, and stages of constriction are shown in figs. 150 (*b*–*f*).

The persistent chromatin granule increases in size, and becomes a conspicuous object in the otherwise lightly-staining zoospore. The cytoplasm meanwhile has become dense, and in many cases vacuoles are no longer discernible. As the zoospore approaches maturity, however, it apparently absorbs water, and a marked expansion takes place (fig. 151). Large vacuoles are now formed, and the cytoplasm becomes confined to strands, whose delicacy increases as the expansion continues; eventually, there is one large central vacuole, round which lie the slender strands of protoplasm. The single chromatin granule meanwhile remains near the periphery of the zoospore, and continues to enlarge. Before maturation, however, its homogeneous appearance is lost, and it becomes resolved into several small granules, while a clear area develops in their midst (fig. 152). In slightly older zoospores, a blepharoplast, with a thread connecting it to the nucleus, becomes visible, but its origin could not be determined (fig. 153). The blepharoplast, as in the soral zoospore, lies at the end of the body, opposite the nucleus, and the thread follows a curved path round the central vacuole. The zoospores are in this condition when they are discharged from the sporangium.

The Appearance of the Original Nucleus of the Resting Sporangium during the Later Stages of Development of the Zoospores.—After the sporangium has reached a stage similar to that shown in fig. 143 the chromatin in the vacuole as before, becomes completely dissolved. The size of the nucleus by this time is much reduced but its spherical form is still retained. On the disappearance of the chromatin of the vacuole the superficial portions of the linin matrix become diffuse and its substance is then gradually distributed in reticulate form throughout the vacuole. While this

distribution is in progress the matrix in many cases becomes separated from the nucleolus. As the linin reticulum is being formed the spaces in it are at first wide (fig. 154), but as distribution continues the width of the mesh is increased (fig. 155), and finally the vacuole is filled with a dense, homogeneous linin substance in which no reticulate structure can be detected. After each of the last few nucleolar discharges the nucleolus may remain in a vacuolated condition, but such vacuoles as are formed are never as large as those which arise after the discharges from the nucleolus of the prosoral nucleus. When the linin is uniformly distributed through the nuclear vacuole an occasional chromatin granule may be given off from the nucleolus, but the amount of chromatin in the nucleolus has been so reduced during the last few discharges that the nucleolus eventually remains as a scarcely perceptible, achromatic sphere usually lying excentrically in the nucleus.

The Formation of the Outermost Layer of the Wall of the Resting Sporangium.—When discussing the epispore of the resting sporangium JOHNSON (17) expressed the opinion that it is formed from "the residual contents of the host cell when not also from its cell wall as well." PERCIVAL (35) on the other hand thought its origin was to be referred to the cell walls alone, and this theory was supported by BALLY (1) who attributed its formation to the liquification of the original cellulose wall of the host cell.

No evidence could be found for BALLY'S suggestion that heavy liquefication of the host cell wall is mainly responsible for the formation of the membrane. On the contrary it is believed that it owes its origin to a change in the constitution of the contents of the host cell, and that it therefore does not consist of a single substance. The greater part of the membrane is formed from the sap contents of the host cell, but at the junction, with the true membrane of the sporangium at its inner surface and with the host cell walls at its outer, the host protoplasm also participates in its formation.

The stages in the deposition of the membrane can be best followed in material which has been fixed in an osmic acid solution and has not been bleached. In bleached material the cell sap in the early stages of membrane formation does not stain readily and as it is at that time naturally colourless, satisfactory stages cannot be obtained. In unbleached material, however, the peculiarly refractive appearance of the cell sap as its constitution begins to change serves as an indication of the progress of the deposition. The description of the process which will be given refers to unbleached material.

Before the outermost membrane is formed the sporangium usually occupies a more or less central position in the cell, and is surrounded by a single layer of large vacuoles separated by delicate layers of protoplasm. A thin layer of protoplasm immediately covers the surface of the sporangium and the walls of the host cell. As the protoplasm dies disintegration products of an oily nature are formed in the vacuoles, and these then appear as dark globules on the surface of the sporangium and of the host wall

(fig. 156). On the sporangium these globules are so numerous that they lie almost in contact with one another.

During the formation of these globules a change has taken place in the appearance of the wide portion of the cell occupied by the sap. The vacuoles are no longer clear but are filled with a substance—apparently gelatinous—which in fixed material has a peculiar milk-white sheen (fig. 157). The sap now forms a jelly and, in the process, sporangium, dead protoplasm, vacuolar region, and host cell wall are united into a firm whole. The milky appearance is now replaced by a clear translucence, and a faint yellow shade becomes discernible in the newly-formed membrane. The colour changes gradually to the deep orange characteristic of the outermost layer of the mature sporangial membrane, but the disintegration globules remain unchanged at its inner and outer surfaces (fig. 158). Owing to the presence of these globules the membrane of the sporangium when examined in surface view seems to be perforated by numberless holes. Sometimes the epispore is completely broken away in the subsequent development of the sporangium. Usually, however, it persists until the time of zoospore liberation, and it is possible that the spaces in the dead cytoplasm between the epispore and the true membrane originally occupied by the oily disintegration globules, are of service in retaining moisture.

The Division of the Infected Host Cell.—It is always actively dividing regions of the host plant which become infected, and as BALLY (1) first stated it is owing to the repeated division of the infection cell that the sporangium becomes deeply placed in the tumour. As, however, he figures only isolated cases of host cell division, a more connected series of events will be described in the present instance.

The zygotes often congregate in such numbers that the majority of the epidermal cells of a given region become infected. Moreover, infection of the different cells is almost simultaneous. After infection has taken place it is usual to find that a large proportion of the epidermal cells divide within a short time of one another, and epidermal cell-division is thus normally widespread. As the result of this division most of the parasites lie in the inner (*i.e.*, lower) of the daughter cells. These infected sub-epidermal cells, however, do not divide so regularly, and in their case cells in which division is taking place are not often to be found near one another. As division is repeated and the sporangia become more deeply placed, the more rare becomes the simultaneous division of cells containing parasites.

In fig. 159 is shown a portion of the epidermis of the host tissue when the wide-spread first division is taking place. In two cells the new walls have been formed parallel to the surface of the tumour, and in a third the wall is set obliquely to the surface. Occasionally the wall is formed at right angles to the surface; when this occurs in cases of multiple infection the parasites are distributed laterally but not buried. In fig. 160 are four adjacent epidermal cells, each containing several parasites of different sizes, but division of the host cell has not yet taken place. Examples of the division of epidermal host cells in which the nuclei are in prophase, metaphase,

and telophase respectively, are shown in figs. 161, 162 and 163. Division of a sub-epidermal cell which was one of the daughter cells formed at the first division after infection may be seen in fig. 164. In this example the wall of the second division, as so frequently happens, will be set obliquely to that of the first division. Division (prophase) of a deeply placed host cell is shown in fig. 165.

Usually, only one maturing resting sporangium occurs in each host cell. Nevertheless, cases of multiple infection of epidermal cells are more common than cases of single infection. Under normal circumstances, division of the infected cell is repeated until the distribution of the parasites through the host tissue is effected. In figs. 166*a*, *b*, which are drawn from adjacent sections of one cell, six parasites can be seen, and, of these, three lie in the inner daughter cell and three remain in the outer. If parasites are left in the epidermal daughter cell, as in this case, or if it should be infected a second time, the cell will continue to divide until the parasites are removed to the tissue below. Thus the final deep position of the parasites is usually due to the repeated divisions of all the daughter cells.

Treatment of the Resting Sporangium and its Relation to Maturation.—The germination of the resting sporangium was secured in potato solutions by JOHNSON (17), and, although empty sporangial cases were observed in those in weak sugar solutions, it was believed that the zoospores were liberated only when the sporangia had developed in contact with, or in decoctions of, the host plant. PERCIVAL (35), however, found that material which had been saved from the previous autumn germinated in spring when suspended in drops of water for five or six days. In the absence of details of their treatment during the winter, however, it is presumed that the sporangia were retained in the decaying tumour, and evidence of the complete development of the sporangia in water was therefore lacking. In the course of the present investigation, it has been found, however, that sporangia removed early in the season from still healthy tissue, can mature and discharge their zoospores when kept in water alone, the length of the maturation period depending upon the state of development of the sporangia when removed. The period for those taken from living tumours soon after the formation of the outermost (sporangial) membrane, and therefore to be regarded as young, was 2½ months. Whether the medium was rain water, soil water, or potato solution of not more than 1 per cent., made very little difference, as the longest and shortest times taken for discharge differed only by about ten days. Rain water, however, produced the quickest germination.

Ripe sporangia may be secured by the simple method of leaving them in the tumour and moistening them from time to time. But, when the progress of the sporangia is to be followed from day to day, isolation is necessary. It was found more convenient to work with large numbers and risk the loss of a certain percentage than to preserve a small number more carefully. Each batch accordingly contained thousands of isolated sporangia, and, although it might seem that great

labour was necessary to secure so many, they are so well protected by thick membranes that the labour of collection is comparatively slight. The tissue of the tumour was teased as finely as possible, and then moistened and rubbed lightly with the finger in a mortar, until the pieces were reduced to about the size of coarse sand grains. Sporangia separate from tissue more readily than cell from cell, and upon this fact the following rubbing and filtering processes depend. By filtering through muslin, the larger pieces of tissue were held back, and the filtrate, containing groups of sporangia, was poured into a funnel covered with ordinary silk. This was then spread on a flat dish, and the sporangia were again rubbed with the finger. By this means nearly all the sporangia were rendered free, and the mesh of the silk was sufficiently narrow to permit their passage on to the glass below, while the larger fragments of tissue were held back. Sediment of the same size as the sporangia was worked through with the latter, but most of it could be removed by centrifuging. After this drastic treatment, groups containing two or three sporangia might still remain, but fully 90 per cent. of the material consisted of isolated sporangia. No attempt was made to keep the sporangia sterile, as comparative freedom from bacteria was all that was required, and this was secured by repeated centrifuging in fresh solutions.

No difference was perceptible in the rates of development of cultures kept in diffuse light and in darkness, but strong illumination was avoided. Development continued between the temperatures of 9° C. and 18° C., the optimum lying between 12° C. and 14° C. In some cases the sporangia were immersed in the media, but usually they were kept only moistened, by being placed on germinating dishes standing in a little solution, or on boulting silk which was kept wetted with the media. The silk afforded a convenient means of noting rapidly the condition of the sporangia, for it could be spread upon a glass plate, and the sporangia inspected directly under the microscope.

From time to time sporangia were fixed, and large numbers were used. They were fixed under reduced pressure in the usual way, and washed in changes of water. A small portion was then cut from a test-tube about $\frac{3}{4}$ inch from the open end. A piece of boulting silk was drawn tightly across the rimmed end and secured by tying. It was moistened, and the sporangia were then gradually poured into it. When the water drained away, a thin layer of very weak agar solution, which had cooled as much as possible, was poured over the sporangia. Just as it solidified, the fastenings were cut, the glass removed, and the silk gently drawn from the agar. The sporangia were retained in the lower portion of the film, and the latter, after being cut into pieces of a suitable size, was dehydrated, imbedded, and sectioned in the usual way.

The Discharge of the Zoospores.

Shortly before the maturation of the zoospores the sporangium enlarges rapidly, and its diameter is often increased by as much as 50 per cent. The outer membranes are now quite soft and much distended, and the hyaline appearance of the innermost membrane becomes most striking (fig. 167). The last stage of development results in the wider spacing of the zoospores, during which processes the full length of the cilium is attained. As the zoospores mature, one portion of the innermost membrane of the sporangium swells up, forming a conical projection, which extends inwards, sometimes as far as the centre of the sporangium (fig. 168). The volume occupied by the zoospores is in consequence much reduced, and great pressure is exerted on the already softened outer membranes. Eventually the sporangium bursts, and the slit formed may extend almost round its periphery (fig. 169).

The zoospores are usually in active movement before the sporangium bursts, and this activity may continue for about two hours. When moving within the sporangium they are particularly sensitive to light. On one occasion, a sporangium containing active zoospores was kept in a weak, dark green light for an hour. By the end of that time the zoospores had gradually all come to rest. The brightness of the light was then increased. In five minutes some of the zoospores were moving; in ten minutes movement was general and rapid; and, shortly afterwards, the dashing to and fro of the earlier swarming period was resumed. When the zoospores are free from the sporangium, however, change in the brightness of the illumination has not been found to have such an appreciable effect upon their rate of movement. The activity of the zoospore of the resting sporangium is stated by PERCIVAL (35) to continue for twenty minutes. On several occasions, however, it was found that, provided the temperature did not rise above 15° C., movement continued for fully two hours, at the end of which time those zoospores that were in contact with the host plant entered the epidermis; those, however, which were not so situated, disintegrated. No zoospores were seen to become amœboid, but this condition of the zoospore has been so frequently reported that its occurrence cannot be doubted.

The Structure of the Zoospore.

The zoospore of the resting sporangium is about 2 μ in length and is slightly larger than that of the sorus, but the two closely resemble one another in appearance. The hyaline, main portion of the zoospore narrows at the refractive, anterior end where the nucleus is situated (fig. 171). The cilium is long and slender; the relatively short cilium of the figures of earlier workers is suggestive of immaturity of the zoospore. Several chromatin granules are to be found on the inner surface of the nuclear membrane and connecting them are a few slender strands of linin (fig. 172). The centre of the nucleus, as in the soral zoospore, is quite clear, and no definite nucleolus can be recognised.

The Entry of the Zoospore into the Host Tissue.

The zoospore usually comes to rest upon its side and, as in the soral zoospore, the nucleus, the blepharoplast and the thread connecting the two are all that comprise the nuclear apparatus before entry into the host plant begins (fig. 174). A slight protruberance, however, is soon noticeable on the side of the nucleus nearest the surface of the zoospore in contact with the host epidermis (figs. 175, 176). The projection extends further and further from the nucleus and it becomes correspondingly attenuated, until the final slender form is attained as it reaches the surface (fig. 177). When the nucleus lies close to the surface of contact the projection has not far to travel, and so is not so fine when it reaches its final position (figs. 178, *a–d*). The end of the projection eventually is pressed against the surface of the zoospore and it becomes slightly flattened (fig. 179). Entry into the host tissue then begins at the point of contact of the disc-shaped end of the projection with the host tissue. The cilium has meanwhile been withdrawn just as in the case of the soral zoospore (figs. 178*a*, 180). The granule-like apex of the nuclear projection is the first visible portion of the zoospore to enter, and in very early stages when its substance has not completely passed through the wall it appears as two small dots on either side of the pore (fig. 181). Protoplasm of the zoospore probably envelops the nuclear apparatus as entry is taking place but it cannot be detected in the early stages. In fig. 182, however, a small amount of cytoplasm has passed within the host cell, where it has already assumed a spherical form. In this example the host cell is plasmolysed, and the nucleus of the zoospore may be seen traversing the space between the wall and the protoplasm. Only a small portion of the zoospore remains upon the surface of the epidermis in the two examples of fig. 183, and in both cases the nucleus has almost completed its entry. The stage just after entry is shown in fig. 184; the main portion of the nucleus now occupies a central position in the organism but a portion still extends towards the pore and this will be drawn up before the spherical form characteristic of the transitional stage of the prosoral nucleus is acquired (fig. 185). Chromatin is formed rapidly and the nucleus soon becomes homogeneous, and the parasite in the meantime travels down the cell. A small clear area now forms round the nucleus, and as this vacuole widens, and a few linin strands appear across it, the stage is reached which was chosen as the starting point in the description of the life-cyle of the fungus.

General Discussion.

The Relation of Fungus and Host Plant.

If the fungus enters young host tissue the resulting tumour probably exhibits no trace of the form of the infected organ, although a slight conducting system is usually developed. When the fugus enters older tissue, however, the organ containing the

parasite may exhibit only local malformation, its tissues elsewhere being normally differentiated.

Although the tissue of the tumour differs in structure from that of uninfected regions of the plant yet it retains much of its normal power of reaction. Thus the cuticle on the surface of the tumour does not develop normally, yet when bacteria enter an epidermal cell of the tumour they are cut off by a cork cambium just as in other parts of the plant.

The behaviour of the infected cell varies according to whether it contains a prosorus or a resting sporangium, death being preceded in the one case by enlargement, and in the other by repeated division. When both zygote and zoospore enter one cell the effect due to the prosorus seems to predominate, for the cell enlarges more than usual and then dies without dividing. The more prosori the cell contains the greater is its enlargement.

The nuclei of injured cells of the tumour exhibit in especial degree the reaction to injury described by MIEHE (33)—that of migration into adjoining cells. If a superficial portion of the tumour is removed and left for twenty minutes before being fixed, it will be found that a general migration of nuclei has taken place in the cells adjoining those cut, the direction of movement being usually from the cut surface. On several occasions two nuclei have been observed entering one cell, and one nucleus leaving its cell as a second entered.

The Persistence of the Organism in the Soil from Year to Year.

Several instances have been reported of the infection of normally healthy potato "seed" in fields which were known to have borne previously a diseased potato crop, but in which no potatoes had been planted for eight years or longer. How the organism maintains itself in the absence of the potato plant is at present unknown.

COTTON (5) has succeeded in infecting other species of *Solanum* with wart disease, thus raising the question of the practical importance of the plurality of hosts. Although it is improbable that species of *Solanum* are present in the neighbourhood of the potato fields in sufficient numbers to be of economic importance in the maintenance of the parasite from season to season, yet certain species of *Synchytrium* are parasitic upon so many and such widely diverse host plants (*e.g.*, *S. aureum* reported to occur on 130 species), that it is possible that *S. endobioticum* also may be found in more plants than are at present recognised as its hosts, and that these may be of sufficiently common occurrence to serve as alternate hosts in the absence of the potato plant.

On the other hand it has been stated several times that the zoospore of the resting sporangium, after swimming freely, passes into an amœboid condition. This suggests that the organism may live for a long time sapropytically in the soil and possibly by repeated encystments may tide over many winter periods.

But there is a third possibility. Resting sporangia have been found to mature

and discharge their zoospores when in water alone. But probably in nature a small proportion of sporangia remain undischarged each year. It is doubtful, however, whether after eight years, sufficient would remain ungerminated to cause the widespread infection reported. In these cases the explanation of the "persistence" of the organism probably lies in the introduction, at planting of the second crop, of tubers which were apparently healthy but which really bore free resting sporangia upon their surface.

What is required, therefore, is to discover in the first place whether the organism really persists for a long period in the soil, and, if it should be proved that it does so exist, to discover whether the resting sporangium remains ungerminated, or whether the infection of other host plants, or the assumption of a saprophytic mode of life, is responsible for the maintenance of the fungus.

Immunity from Wart Disease.

It is a well known fact that certain varieties of potato are immune to wart disease, but the cause of this immunity is still to be discovered. In the case of the immunity of certain wheats to *Puccinia glumarum*, Miss MARRYAT (29) has shown that, "though the fungus succeeds in making good its entry and producing hyphæ, further progress is either completely checked by the breaking down and death of the host tissue locally, accompanied by the starvation and death of the parasite," or else, in cases of partial immunity, "a more protracted struggle takes place . . . and the development of the fungus proceeds to a farther point, but is still greatly retarded."

In the varieties of flax immune to *Fusarium Lini*, it was found by TISDALE and JONES (18) that in this case also the fungus succeeds in penetrating the host plant. In the susceptible varieties, the parasite penetrates directly to the vessels of the root. "In the resistant individuals, on the other hand, the invasion advances more slowly, and, before it reaches the vessels, is checked, and permanently walled off by the development of a corky layer."

So far, we have no indication wherein the immunity to wart disease lies. It may be due to a reaction between host and parasite similar to one or other of the above cases. On the other hand, it is possible that infection does not occur at all, possibly because the zoospores are not attracted to immune plants, or because a physical obstacle, such as a thick cuticle, intervenes. It is intended shortly to undertake an investigation of the mutual reaction between the parasite and immune varieties of potato.

Nuclear Reduction.

The fusion of the gametes is a sexual process involving a subsequent nuclear reduction. But, in the stage in which it is natural to expect the reduction to take place—the development of the resting sporangium—mitosis does not occur. A large amount of chromatin, however, is accumulated in the zoospore primordia of the resting sporangium, and the greater part of it is later thrown off, after which

process the typical zoospore nucleus is developed. The zoospore of the resting sporangium produces the sorus, but, in the development of that organ, we fail to find any nuclear division which suggests a reduction. A typical reduction thus appears to be completely absent from the life-cycle. It is therefore concluded that the rejection of chromatin by the primordia of the zoospores of the resting sporangia must be regarded as equivalent to the nuclear reduction of the higher plants, the haploid generation thus starting at the maturation of these zoospores.

The Relation of the Fungus to the Genus Pycnochytrium (DE BARY), SCHRÖT.

The diagnosis of the genus *Pycnochytrium* (DE BARY), given by SCHRÖTER (45) in 'Die Natürlichen Pflanzenfamilien,' reads as follows: "Fruchtkörper endogen, nach vollendeter Reife mit einer festen Haut umgeben. Schwärmsporangiensori nicht unmittelbar aus dem erwachsenen Fruchtkörper gebildet, sondern dadurch, dass sich der Inhalt des Fruchtkörpers durch eine feine Oeffnung entleert und eine dünnwandige Zelle bildet, deren Inhalt in Sporangien zerfällt. Einzelsporangien dicht gelagert, durch den gegenseitigen Druck vielkantig, oft unregelmässig gestaltet; Entleerung durch kurze, warzenartige Mündung; Membran farblos. Schwärmsporen rundlich, mit 1 Cilie. Dauersporangien aus einem erwachsenen Fruchtkörper gebildet; dick Membran, braun." From the account which has just been given of the development of the fungus at present under consideration, it will be seen that it exhibits all the characteristics enumerated by SCHRÖTER in the diagnosis of the genus *Pycnochytrium* (DE BARY), SCHRÖT.

This genus is divided into three sections, the description of one of which reads: "Die Entleerung des ursprünglichen Fruchtkörpers und Ausbildung der Schwärmsporangien erfolgt schon auf der lebenden Nährpfl. in der ursprünglichen Nährzelle. Nebenher werden noch Dauersporangien gebildet, welche eine längere Ruhepause durchmachen."

In this division—called by SCHRÖTER *Mesochytrium*—the present fungus may be included, for in all four points its development follows the description given. In the three species of this section, the contents of the prosorus migrate either in the upward or in the downward direction in the host cell; *Pycnochytrium Succisæ* (DE BARY and WORONIN), SCHRÖT., is the only species in which the migration is upwards. The number of sporangia formed in the sorus is greater in *P. Succisæ* than in *Synchytrium endobioticum;* wide variation in this respect, however, may be seen throughout the genus. The only other major distinction between the two species is that of the colour of the oil in the cytoplasmic vacuoles, that of *Pycnochytrium Succisæ* being orange and of *Synchytrium endobioticum* hyaline. The other sections of the genus *Pycnochytrium*, however, exhibit the same colour distinction, and its extension to the section *Mesochytrium* was to be expected as our knowledge of the genus widened.

Since, as has been shown above, the present species exhibits without exception all

the characteristics of the genus *Pycnochytrium*, the retention of the name *Chrysophlyctis endobiotica* (SCHILB.) is no longer justified. Whether the organism is called by the broad generic name *Synchytrium*, as suggested by PERCIVAL (35), or by SCHRÖTER'S narrower generic term *Pycnochytrium*, depends on whether FISCHER'S or SCHRÖTER'S classification of the *Synchytriaceæ* is accepted. As the reasons with which SCHRÖTER justifies his resolution of the old genus *Synchytrium* into two new genera—one of which, though narrower in scope than the original genus, retains the name *Synchytrium*, while the other is named *Pycnochytrium*—do not seem to be sufficiently valid to warrant the change, the writer prefers to recognise FISCHER'S classification of the wider genus *Synchytrium*. In consequence, as will be seen from the title of the present paper, it is not suggested that the generic name *Synchytrium*, given to the organism by PERCIVAL (35), should be changed to that of *Pycnochytrium* (DE BARY), SCHRÖT.

Sexuality in the Synchytriaceæ.

Although the occurrence of sexuality in the *Synchytriaceæ* has long been suspected, this is the first case in which the actual process of fusion has been observed. But the accounts of early workers (DE BARY (2), WORONIN (50), SCHRÖTER (44)) contain so many references to "giant zoospores" possessed of two cilia—which were probably zygotes—that there is a great possibility that sexual fusion similar to that of the present species will be found to take place in all those members of the (older) genus *Synchytrium*, in which true resting sporangia are formed.

SUMMARY.

The occurrence of sexuality in the *Synchytriaceæ* has long been suspected, but the fusion of motile isogametes recorded in this paper is the first instance in which a sexual process has been observed.

Two forms of reproductive bodies are formed in *Synchytrium endobioticum*, the resting sporangium, and the sorus. The resting sporangium produces zoospores. The sorus produces motile cells, which can behave either as zoospores or as gametes; there is evidence that treatment during the later stage of maturation of the sorus is of influence in determining the sexual or asexual nature of its motile cells. It would seem, however, that the gametes produced as the result of these special conditions during maturation are of facultative nature. It would seem probable also that the gametes fuse only with those of another sporangium.

The zoospores of the resting sporangium are almost oval in form, have a single long posterior cilium, and a bright anterior spot where the nucleus lies. The cilium is attached to a blepharoplast, and a fine chromatic thread joins blepharoplast and nucleus. When infecting the potato plant, the zoospore passes through the outer wall of an epidermal cell of young tissue. Nucleus and cytoplasm are much compressed during entry, but both resume a spherical form as the organism emerges

into the host cell. The body of the organism, from the time of entry to that of the cleavage into sporangia, has, for convenience, been termed the prosorus.

The young prosorus passes to the lower end of the host cell. The cytoplasm shows a fine network and the nucleus appears as a single homogeneous granule. From this granule a nucleus of more typical form arises by the development round it of a clear area across which run linin strands from granule to membrane. The granule may now be spoken of as the nucleolus. Whether the cavity of the nucleus so produced is of nuclear or of cytoplasmic origin could not be determined.

A process of nucleolar discharge sets in; globules, consisting of both chromatin and material, are formed on the nucleolus and their substance is given off into the nuclear cavity; the chromatin is dissolved, while the linin becomes distributed through the nuclear cavity. Three discharges take place at this phase; after the two earlier ones the nucleolus may again become homogeneous; at the close of the second discharge the nucleolus is found in contact with the nuclear membrane.

Prophase of the primary mitosis is considered to begin when the third nucleolar discharge sets in; at the close of this discharge the nuclear cavity is filled with a homogeneous mass of linin. At this stage fine strands, five in number, are to be seen extending from the nucleolus towards the membrane on the opposite side.

The host cell has enlarged and is now dead, while the parasite fills its lower half; the prosorus develops a thick orange outer, and a thin hyaline inner, membrane; great numbers of chromatic granules are present in its cytoplasm. The cytoplasm and nucleus of the prosorus, while surrounded with a delicate membrane, now pass through a pore in the upper region of the outer membrane into the host cell.

Repeated mitosis of the nucleus takes place before the cytoplasm has wholly emerged from the pore. There are five minute spherical chromosomes; it is believed that they originate from a globule given off by the nucleolus. The spindle is intranuclear in origin, the nuclear membrane persists until metaphase. No trace of asters or of centrosomes was found.

After about five nuclear divisions the prosorus segments into from four to nine sporangia, the walls being formed simultaneously and independently of the nuclei. The sporangia remain thin-walled and enclosed in the delicate common membrane. Nuclear divisions continue until 200–300 nuclei are formed in each sporangium. The chromatic granules in the cytoplasm have by now disappeared.

On the absorption of water a small vacuolated area appears round each nucleus, and separating the areas are dense intersecting strands of protoplasm. A chromatic granule, the blepharoplast, lies near the periphery of each area and is connected to the nucleus by a deeply staining thread. Separation of these areas is effected by the formation of the vacuoles in the middle of the dense delimiting strands. The parts so separated, which first appeared as the lighter areas, become the young zoospores. As separation continues the connection between the zoospores is reduced

to a single long strand running from the blepharoplast of one zoospore to the surface of another. Finally the strand becomes free at the end which is not attached to the blepharoplast and thus the cilium is produced.

Repeated division of the host tissue has meanwhile resulted in the formation of a tumour; in addition the epidermal cells in contact with the infected cell have grown outwards into a rosette of elongated refractive cells surrounding the infected cell.

At the time of the cleavage of the prosorus into sporangia the cells lying beneath the parasitised cell divide several times by walls lying almost parallel to the surface of the tumour. At the maturation of the sporangia these cells absorb water and enlarge, exerting pressure upon and raising the infected cell and its rosette above the surface of the tumour; pressure is exerted on the infected cell from within owing to the enlarging sporangia. Under the combined pressures the cell and soral membrane burst and the sporangia become free.

One or two hyaline projections are formed on the surface of the sporangium and through the rupture of one of these the motile cells are liberated. If the motile cell is a zoospore it develops into a sorus; if it is a gamete, it fuses with a similar gamete, probably from another sporangium, and the resulting zygote develops into a resting sporangium. The motile cells from the sorus are smaller than the zoospores of the resting sporangium, but are similar to them in structure.

The gamete nuclei fuse and the cilia shorten before the passage of the zygote into the host takes place. A nuclear projection plays a part in the process of entry.

The zygote after entering passes to the lower end of the host cell. The parasite nucleus appears as a spherical granule of chromatic material round which a clear space develops. The substance of the nucleolus (*i.e.*, the deeply staining granule) is then repeatedly given off into the nuclear vacuole; the chromatin of the discharged substance dissolves, the linin remaining in the vacuole. Soon after the nucleolar discharges begin chromatic "granules" appear in the cytoplasm of the parasite, and at the same time a cell wall is formed round the body of the young resting sporangium. The "granules" enlarge and show a differentiation into chromatic and achromatic material; these "granules" are the primordia of zoospores. The chromatic material, which is arranged in irregular strands near the periphery of the primordia, begins to project from the surface at several points, and a large proportion of it is then given off into the cytoplasm of the sporangium. Eventually a single chromatin granule is left in each young zoospore; from this granule the nucleus of the mature zoospore arises. A blepharosplast, with a deeply staining strand connecting it to the nucleus, is now formed.

The sporangial membrane shows three layers. The two inner layers of the membrane are derived solely from the parasite; the outer layer is formed from material derived from the disorganising host cell. The rupture of the resting sporangium results from the swelling of the innermost hyaline layer of the membrane.

In the accounts of early workers upon the *Synchytriaceæ*, and particularly in those

of De Bary (2), Woronin (50), and Schröter (44), so many references are made to *giant zoospores possessed of two cilia* that there seems to be a great probability of the occurrence of a sexual fusion, similar to that of the present species, in all those members in which true resting sporangia are formed.

The present species is shown to exhibit all the characteristics of a true *Synchytrium*, being closely allied to *S. succisæ*, from which it differs in the colour of the contents of the sorus and of the resting sporangium, and in the number of sporangia formed in the sorus. As Schröter's distinction between *Pycnochytrium* and *Synchytrium* does not appear to be a satisfactory one it seems preferable to include all the species of these two genera in Fischer's original genus *Synchytrium*, and the name *Synchytrium endobioticum* is accordingly retained.

In conclusion, the writer would express her indebtedness to Prof. V. H. Blackman, at whose suggestion the present investigation was undertaken, for his most helpful criticism throughout the course of the work.

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DESCRIPTION OF PLATES.

PLATE 12.

Sorus: General.

Fig. 2.*—Young tumours bearing sori only; length of shoot, 7 mm.

Fig. 3.—Surface view of group of immature sori; a single depressed host cell in the centre of each rosette. Drawn from a photograph. × about 150.

Fig. 4.—Rosette containing enlarging host cell; rosette cells not yet divided. × 170.

Fig. 5.—Rosette cells divided; the prosoral contents have migrated obliquely; the outline of the host cell is indicated by a dotted line. × 170.

Fig. 6.—Side view of young rosette; host cell indicated by dotted lines. × 170.

Fig. 7.—Surface view of mature sorus showing three sporangia in a much enlarged host cell. × 170.

Fig. 8.—Surface view of ruptured host cell; three young prosori lie on the left of the rosette and three young resting sporangia on the right. × 170.

Prosorus.

Fig. 9.—Very young prosorus passing down towards the nucleus of the host cell; the cytoplasm of the prosorus is arranged in radiating strands, and the nucleus is still slightly irregular in shape. × 1650.

Fig. 10*a*.—Enlarging prosorus. The nucleus is still irregular in shape while the cytoplasm shows a reticulate arrangement. × 2000.

Fig. 10*b*.—The nucleus has much enlarged, but traces of the granules of the zoospore nucleus can still be seen. × 2000.

Fig. 10*c*.—The nucleus is spherical and homogeneously dark and lies free in the cytoplasm. × 2000.

Fig. 10*d*.—A clear area has arisen round the homogeneous granule. × 2000.

Fig. 11.—Young prosorus in an enlarged host cell. The contents of the host cell are much vacuolated. The nucleus of the prosorus consists of a central nucleolus and a large nuclear cavity containing chromatin which has arisen from a prévious nucleolar discharge. The enlarged host nucleus rests upon the prosorus. × 400.

Fig. 12.—Young prosorus with minute chromatin granules in its cytoplasm, and a large nucleus in the cavity of which chromatin is undergoing solution. Disorganisation of the host-nucleus is beginning. On the right-hand side, the cell adjoining the host cell has grown up to form the rosette. A rosette cell is absent from the left-hand side as there another host cell adjoins. × 400.

* For fig. 1 see text-fig. on p. 412.

Fig. 13.—Prosorus shortly before the migration of its contents. It has a firm membrane and numerous chromatin granules in the cytoplasm. The nucleolus lies upon the nuclear membrane, and extending from it across the now densely filled nuclear cavity are several long linin threads. Lying immediately beneath the membrane in the upper part of the prosorus is the dense mass of cytoplasm which will begin the process of migration, and above which the pore will be formed. × 400.

Prosorus: Second Nucleolar Discharge.

Fig. 14.—Nucleus before discharge has begun. There is a large nuclear cavity, suspended at the centre of which, by a few linin strands, is the nucleolus. No chromatin is present in the nuclear cavity. × 800.

Fig. 14*a*.—Nucleus after first discharge; the nucleolus is vacuolated; linin and chromatin are present in the nuclear cavity. × 800.

Fig. 15.—Beginning of the second nucleolar discharge. Globules of chromatin are forming on the surface of the nucleolus. × 800.

Fig. 16.—The substance of the nucleolar globules has become opened up into an irregular interwoven mass of chromatin extending across the nuclear cavity. Occasional globules, which will be given off independently of a general discharge, are already to be seen upon the nucleolus. × 800.

Fig. 17.—The chromatin discharged from the nucleolus has undergone partial solution and now occurs as isolated granules, while the linin is becoming evident. × 800.

Fig. 18*a*.—The substance of the second discharge has been given off in a single globule, and a large vacuole has consequently been formed in the nucleolus. No chromatin remains in the nuclear cavity. The linin can be traced to the mouth of the nucleolar vacuole. × 800.

Fig. 18*b*.—The linin is becoming evenly distributed. The nucleolus contains several vacuoles. × 800.

The Third Nucleolar Discharge.

Fig. 19.—The globules of the third discharge are forming. The linin is now more or less evenly distributed, and the nucleolus is once more homogeneous in appearance. × 800.

Fig. 20.—A lightly staining, finely vacuolated nucleolus bearing the numerous globules characteristic of the third discharge. The nucleus has been relatively inactive and the chromatin in the cavity is not completely dissolved. The nucleolus now rests on the membrane. × 800.

Fig. 21.—The chromatin is being transferred from the globules to the long heavy strands. The linin arising from the previous discharges is uniformly distributed and almost homogeneous, only a slight trace of the earlier reticulum remaining. × 800.

Fig. 21*a*.—An early stage of transference of chromatin from globules to strands. × 1650.

Fig. 22.—Transference of the chromatin of a globule to strands; chromatin solution has begun. × 1650.

Fig. 23.—Chromatin solution advanced; granules isolated on the several distinct linin strands; the nucleolus is much vacuolated. × 800.

Fig. 24.—All the chromatin has disappeared from the strands which are now five in number and delicate in structure. × 800.

Fig. 24*a*.—A nucleus in which the chromatin has become separate from the strands and now lies as granules near the membrane. The nucleolus in this example has contracted before migration has taken place. × 800.

Fig. 25.—A nucleus in which the third nucleolar discharge has followed quickly upon the second; the linin in the cavity, due to the second discharge, has not yet lost its reticulate arrangement. × 800.

Fig. 26*a*.—Formation of the globules for a discharge in a young homogeneous nucleolus; their substance is drawn from regions near the surface, and the vacuole formed is not much larger than the globule. × 1650.

Fig. 26*b*.—Formation of globules in a vacuolated nucleolus. The globules are formed deep within the nucleolus and are much smaller than the vacuolar region from which they have drawn their substance. × 1650.

The Passage of the Prosoral Contents into the Host Cell.

Fig. 27.—Prosorus before the passage of the contents begins. The protoplasm beneath that portion of the membrane where the pore will be formed has become dense and free from chromatin granules. A slight cap of dense protoplasm surmounts the nucleus. × 800.

Fig. 28*a*.—The beginning of the passage. The dense portion of protoplasm alone has passed out; the edge of the outer membrane at the pore is turned up; the small portion of the inner lining is expanding to keep pace with the outflowing protoplasm which it will continue to cover; a slight deposition of the dead host cell contents can be seen on the outer membrane. × 800.

Fig. 28*b*.—The pore at a later stage; the edge is recurved; the heavy host cell deposit on the membrane is shaded, the outer membrane itself is represented as a clear envelope; the inner membrane can be traced from outside through the pore. × 1650.

Fig. 29.—Two prosori in a much enlarged host cell discharging at the same time, obliquely, and in opposite directions. × 400.

Fig. 30.—The nucleus has begun to move and is preceded by a dense strand of protoplasm originating from the cap which previously surmounted it; the dense protoplasm which lay beneath the pore is now spread out round the periphery of the expanding protrusion. × 400.

Fig. 31.—The apex of the nucleus has reached the pore, but the lower end has not yet left its original position. × 400.

Fig. 32.—The apex of the nucleus is through the pore and is already expanding as it continues to move forward; vacuoles have been formed round the periphery of the protoplasm within the original membrane. × 400.

Fig. 33.—The nucleus is more than half through the pore (slightly plasmolysed). × 400.

PLATE 13.

Fig. 34.—The nucleus reconstituted in its final position; the fine linin strands are evident and the nucleolus is budding. The vacuoles round the cytoplasm in the original envelope are now more conspicuous; the section is tangential to the host cell. × 400.

Fig. 35.—Two nuclear divisions have occurred, yet a few strands of protoplasm still remain in the original envelope; a vacuole has expanded into the region of the discharged protoplasm. The section is slightly tangential to the host cell. × 400.

Fig. 36.—Stages in the formation of the vacuoles at the periphery of the protoplasm in the original envelope :—

(*a*) Early; longitudinal section through the pore. × 400.
(*b*) Late; longitudinal section through the pore. × 400.
(*c*) Fairly late; section transverse to axis which passes through the pore. × 400.

Mitosis of the Primary Nucleus. Late Prophase.

(Prophase begins with the third nucleolar discharge before the passage of the prosoral contents.)

Fig. 37.—The five linin threads are loosely coiled together near the nucleolus; extremely delicate strands connect them to the nuclear membrane; the vacuolar region is filled with the homogeneous linin from the nucleolar discharges. × 1650.

Fig. 38.—The linin strands beginning to straighten. × 1650.

Fig. 39.—The linin strands are beginning to assume parallel positions across the centre of the nucleus; the nucleolus which is budding will pass out to one side. × 1650.

Fig. 40.—Three strands almost parallel; the nucleolus has passed to one side and bears a large globule, possibly for the formation of the chromosomes. × 1650.

Metaphase.

Fig. 41.—The spindle is long and slender, the chromosomes are five in number, spherical and minute; the nucleolus and two small chromatin granules are visible; the nuclear membrane still exists. × 1650.

Mitosis of the Secondary Nuclei of Prosorus and Sorus. Resting Stage.

Fig. 42.—Five nuclei of different sizes in the resting condition; the excentric nucleolus is evident only in the larger nuclei; the clear central cavity is traversed by several linin strands. × 1650.

Prophase.

Fig. 43.—The peripheral chromatin granules have almost disappeared, and a linin reticulum is formed across the nuclear cavity. × 1650.

Fig. 44.—The nucleolus is budding freely, the granules will, however, disappear later. Linin strands of the future spindle are evident and the nuclear cavity is homogeneous. × 1650.

Fig. 45.—Slightly later stage than fig. 44; the granules have disappeared; linin strands now distinct. × 1650.

Figs. 46*a*, *b*, *c*.—Three stages in the giving off of the large globule from which the chromosomes are probably formed. × 1650.

Fig. 47.—The five chromosomes; the spindle threads not evident. × 1650.

Metaphase.

Fig. 48.—The spindle is formed, and three of the five chromosomes are shown; the nuclear membrane still persists; the nuclear cavity is filled with homogeneous linin; the nucleolus lies to the side of the spindle. × 1650.

Figs. 49*a*, *b*.—The five chromosomes are evident. × 1650.

Anaphase.

Fig. 50.—Early anaphase; the daughter chromosomes have just separated; elongation of the spindle has begun. × 1650.

Figs. 51*a* and *b*.—Two stages in the separation of the daughter chromosomes; the portion of the spindle between the groups is maintained at its full width until late. × 1650.

Telophase.

Fig. 52*a*.—The elongated spindles have narrowed to a darkly staining thread; the chromosomes are uniting to form a chromatic granule. × 1650.

Fig. 52*b*.—The end of the spindle seems to be included in the chromatic granule formed from the uniting daughter chromosomes; the vacuole has been formed round the granule but is still narrow. × 1650.

Fig. 53.—The vacuole has widened, the granule is homogeneous, and a few linin strands have been formed; the remains of the spindle are still evident. × 1650.

Fig. 54.—The evolution of the resting nucleus; granules are being given off from the nucleolus in fig. 54*b*. × 1650.

Fig. 55.—As fig. 54, but the nuclei are smaller. In figs. *b* and *c* globules are being given off from the nucleolus in the formation of peripheral granules of the nucleus. × 1650.

Segmentation of the Prosorus.

Fig. 56.—Early stage in segmentation; the protoplasm is denser along the course of the future walls. × 800.

Fig. 57.—An undulating, deeply staining layer of protoplasm is visible, down the middle of which the membrane will be formed. × 2300.

Fig. 58.—The undulating layer has become straighter and in the middle a delicate membrane is differentiated. × 2300.

Fig. 59.—The newly formed membranes intersect one another at definite angles. × 2300.

The Sorus.

Fig. 60.—A sorus showing five sporangia shortly after the formation of the cleavage membranes; the nuclei already show a difference of one generation. The section is tangential to the host cell; slightly plasmolysed. × 400.

Fig. 61.—Longitudinal section of host cell showing two sporangia of a sorus above, and an empty prosoral membrane below; the nuclei have undergone several divisions. × 400.

Fig. 61*a*.—The nuclei have become further reduced in size, and are all of the same size. × 400.

Fig. 62.—A portion of a sporangium shortly before the delimitation of the zoospores. The nuclei have a clear central cavity and one or two peripheral chromatin granules, but a nucleolus cannot be distinguished. The cytoplasm is now dense and no chromatic granules can be seen in it. × 1650.

The Formation of the Zoospores.

Fig. 63.—The zoospore areas are delimited and some are becoming separated through the formation of vacuoles down the middle of the delimiting strands. The nucleus, blepharoplast, and deeply staining strand connecting the two can be seen. × 2300.

Fig. 64.—The separation is more advanced than in fig. 63. × 2300.

Fig. 65.—The strands which persist are now unequal in length; the zoospores are enlarging. × 2300.

Fig. 66.—The strands are joined at one end to a blepharoplast and at the other (upper) end to the body of a zoospore (but not to its blepharoplast) or to similar long threads. On separating the threads become the cilia of the zoospores. × 2300.

The Liberation of the Sporangia.

Fig. 67.—The cells beneath the host cell have enlarged and exerted pressure upon the host cell from below. × 170.

The Formation of the Rosette.

Fig. 68.—The prosorus has already reached a moderate size but no elongation has yet taken place in the epidermal cells immediately adjoining the host cell. × 170.

Fig. 69.—The prosorus has begun to discharge and the adjoining cells have become elongated. × 170.

Fig. 70.—The prosorus has completed its passage and the epidermal cells have grown up with the enlarging host cell, leaving however a small uncovered area on the surface of the host cell. × 170.

Fig. 71.—The cells of the rosette after the liberation of the sporangia. A portion of the original envelope of the prosorus is still attached to the host cell wall. × 170.

The Discharge and Structure of the Zoospores.

Fig. 72.—A sporangium swelling prior to the discharge of the zoospores; a hyaline projection is formed. × 780.

Fig. 72*a*.—Three mature sporangia at the base of a host cell; zoospores are already liberated in the cell; the common soral envelope surrounds the sporangia; a small portion, in section, of the original envelope of the prosorus is to be seen, against one wall of the cell. × 400.

PLATE 14.

Fig. 73.—Living zoospores; the refractive anterior spot denotes the position of the nucleus. × 780.

Fig. 74*a*.—Zoospore showing nucleus, blepharoplast, and the strand connecting it to the nucleus; the nucleus has a clear central cavity and a few peripheral chromatin granules, but no distinct nucleolus. × 1650.

Fig. 74*b*.—Fixed zoospore, but unbleached and stained with HEIDENHAIN'S iron-alum-hæmatoxylin, showing nucleus, blepharoplast, and fat globules adjoining the nucleus. × 1650.

Entry of the Soral Zoospore.

Figs. 75*a–f*.—Stages in the retraction of the cilium. × 2000.

Fig. 76*a*.—Zoospore on the surface of the host plant; the projection just leaving the nucleus. × 2000.

Fig. 76*b*.—The projection has advanced almost to the surface of the zoospore in contact with the host cell. × 2000.

Fig. 77.—The projection from the nucleus has reached the surface of the cell, and the portion between the apical granule and the nucleus now appears as a deeply staining thread; the blepharoplast is also visible. × 2000.

Fig. 78.—The earliest stage of entry; the apical granule of the nuclear projection is half way through the host wall; surface view. × 2000.

Fig. 79.—The apical granule of nuclear projection is within the host wall, and the strand connecting it to the nucleus without can be seen. × 2000.

Fig. 80.—Stage of entry slightly later than fig. 79. × 2000.

Fig. 81.—Surface view of entering zoospore; a small portion of the cytoplasm has entered in addition to the apical portion of the nuclear projection. × 2000.

Fig. 82.—The main body of the nucleus is elongating towards the entry pore. × 2000.

Fig. 83.—The nucleus has almost completed its entry. × 2000.

Figs. 84 and 85.—The nucleus is wholly within the host wall and only a vesicle of cytoplasm remains without. × 2000.

Fig. 86.—Both nucleus and cytoplasm have completely entered, but the nucleus still remains connected to the pore, although its main part has already resumed its spherical form. × 2000.

Fig. 87.—The zoospore (prosorus) beginning to travel down the host cell close to a side wall. × 2000.

Fig. 88.—As fig. 87; slightly older prosorus with reticulately arranged cytoplasm. × 2000.

Fig. 89.—The nucleus of the prosorus is now enlarged and homogeneous and a clear area is appearing round it. × 400 and 2000.

Fig. 90.—The prosorus further enlarged; the typical nuclear cavity is now formed, and the chromatic globule is suspended in the centre. × 2000.

The Fusion Process.

Fig. 91*a*.—Two gametes approaching each other. × 2000.

Fig. 91*b*.—The gametes have touched, but face in slightly different directions in the two examples. × 2000.

Fig. 92.—The gametes are pressed together, but the membranes are still distinct; unbleached, stained with HEIDENHAIN'S iron-alum-hæmatoxylin. × 1650.

Fig. 93.—The membranes have disappeared from the region of contact and only a slightly denser strand of protoplasm marks the line of union. × 2000.

Figs. 94*a–e*.—Stages in the approach of the gamete nuclei. × 2000.

Figs. 95*a*, *b*, *c*.—The nuclei become pressed together and the surfaces in contact flatten. × 2000.

Figs. 96*a*, *b*.—Stages in the movement of the chromatin from the joined membranes to the poles of the nuclei. × 2000.

Fig. 97.—The polar accumulation of chromatin becoming distributed round the outer surfaces of the nuclei; the joined membranes are now free from chromatin. × 2000.

Fig. 98.—The fusion nucleus; the chromatin is arranged round the periphery in a broad irregular strand. × 2000.

Fig. 99.—The chromatin of the fusion nucleus is becoming resolved into granules. × 2000.

Fig. 100.—The fusion nucleus now has isolated peripheral chromatin granules and a single strand of linin. × 2000.

Figs. 101*a*, *b*.—The mature zygote nucleus; a more intricate system of linin threads has now developed. × 2000.

Fig. 102.—An aggregation of zygotes in a depression on the surface of the tumour; two ruptured sori lie near but are not shown in the figure. The figure is drawn from a section. There were as many as 184 zygotes in this depression. × 780.

Fig. 103.—Gametes and zygotes in a channel between three rosette cells; on the lower cell there are four mature zygotes and several gametes; above are different stages in the approach of the zygote nuclei. × 1650.

Fig. 104.—From a section 4μ in thickness, showing in what large numbers the zygotes cluster on the surface; the large cell to the right-hand side is a crumpled rosette cell. × 800.

The Entry of the Zygote.

Figs. 105*a*–*g*.—Stages of contraction of the zygote cilia; the nuclei are at different stages of fusion; in fig. *e* the zygote is already beginning to enter. × 2000.

Figs. 106*a*, *b*, *c*.—Three stages in the development of the nuclear projection; in two of the zygotes blepharoplasts are visible. × 2000.

Fig. 107.—The nuclear projection has reached the surface of the zygote; chromatin is accumulating in the apical granule; plasmolysis has occurred. × 2000.

PLATE 15.

Fig. 108.—Surface view of early stage of zygote entry; the apical granule of the nuclear projection is half way through the host wall. × 2000.

Fig. 109.—Three stages of early entry; the apical granule has wholly passed through the wall; in two cases blepharoplasts are visible. × 2000.

Fig. 110.—Slightly later stage than those of fig. 109; a little cytoplasm is now evident within the host cell. × 2000.

Fig. 111.—The portion of the nucleus which has entered is expanding into a structure of irregular form: both blepharoplasts and the strands connecting them to the nucleus are visible. × 2000.

Fig. 112.—The nucleus of the zygote lies unusually close to the surface of contact of zygote and host plant; no nuclear projection can be seen. × 2000.

Fig. 113.—Two later stages in the entry of nucleus and cytoplasm. × 2000.

Figs. 114*a*, *b*.—The zygote nucleus as it emerges from the pore is very irregular in shape. × 2000.

Fig. 115.—The nucleus is becoming spherical, but a portion is still extended towards the point of entry. × 2000.

Figs. 116-118.—Three late stages in the entry of the cytoplasm of the zygote after the nucleus has entered. × 2000.

Fig. 119.—The last portion of the cytoplasm to enter appears as a small granule outside the cell; plasmolysis has occurred. × 2000.

General Appearance of the Resting Sporangium in the Tumour.

Fig. 120.—Epidermal region of tumour bearing resting sporangia in a fairly advanced state of development; the zoospore-primordia are large; the original nucleus of the sporangium is shrunken and the epispore has been formed. × 170.

Fig. 121.—A very young resting sporangium which has passed down the host cell as far as the nucleus. × 400.

Fig. 122.—The young sporangium is now on the lower side of the host nucleus; a few chromatin granules have been given off into the cavity of the nucleus of the sporangium; host cell division has not yet taken place. × 400.

Fig. 123.—Host cell division has taken place and the sporangium lies in a sub-epidermal cell; chromatin granules have been formed in the cytoplasm of the sporangium, and a small amount of linin may be seen in the cavity of the nucleus. × 400.

Fig. 124.—The formation of the true membrane of the sporangium has begun; the nucleus has enlarged and contains more linin. × 400.

Fig. 125.—A second division of the host cell has occurred and the sporangium now lies in the third layer of cells; the contents of the host cell are reduced to a thin layer of protoplasm covering the sporangium and the host walls; the granules in the cytoplasm of the sporangium are conspicuously large; the membrane has almost reached its final thickness. × 400.

Fig. 126.—The dead contents of the host cell have been deposited as an epispore upon the sporangium; the wide clear central region of the epispore is formed from the sap region of the host cell; the more deeply staining inner and outer surfaces of the epispore arise from the host protoplasm. The outer layer of the true sporangial membrane is thick and is shown lightly shaded in the figure; the inner layer is too thin to be visible under the present magnification; the nucleus is shrunken and irregular in form, the nucleolus is colourless, and the nuclear cavity is filled with linin; the zoospore-primordia have enlarged greatly. × 400.

The Development of the Resting Sporangium.

Fig. 127.—A very young stage; the nucleus is a spherical chromatin globule lying free in the cytoplasm. × 1650.

Fig. 128.—A clear area has appeared round the chromatin globule. × 1650.

Fig. 129.—The clear area round the chromatin globule has widened, and linin threads pass across it; a definite nuclear membrane may be assumed to exist from this stage onwards. × 1650.

Fig. 130.—A stage immediately before the first nucleolar discharge begins; the nucleolus stains deeply at its periphery. × 1650.

Fig. 131.—An early stage of the first nucleolar discharge; small granules have been formed on a limited area of the nucleolus. × 1650.

Fig. 132.—The first nucleolar discharge; the granules have increased in size and now form a compact mass. × 1650.

Fig. 133.—The first nucleolar discharge; the chromatin of the projection is being dissolved and a linin-like substance is revealed between the isolated granules. × 1650.

Fig. 134.—A slightly later stage than fig. 133; the nucleolus stains deeply but it has been reduced to an oval shape during the discharge. × 1650.

Fig. 135.—First nucleolar discharge; the chromatin has completely disappeared from the nuclear cavity and only linin remains. × 1650.

Fig. 135*a*.—The second nucleolar discharge. Stages similar to those of figs. 131–135. × 1650.

Fig. 136.—The formation of the chromatic granules in the cytoplasm of the sporangium. *a*. Four large condensation areas are shown; minute granules may be detected on their strands. × 2000. *b*. New, smaller areas have appeared between the original ones. × 2000.

Fig. 137.—The third nucleolar discharge beginning; the granules have developed on the nucleolus; chromatic granules are now present in the cytoplasm of the sporangium. × 800.

Fig. 138.—The sporangial membrane although thin is now distinct; the nucleolus is still undergoing the third discharge and the chromatin of the projection is becoming dissolved; the nucleus has now reached its maximum size. × 800.

Fig. 139.—The third nucleolar discharge is completed; the chromatin has dissolved and only linin remains; the granules in the cytoplasm are larger and more numerous than in previous figures, and the membrane is denser. × 800.

Fig. 140.—Slightly later stage than that of fig. 139; the granules in the cytoplasm are more numerous, and the membrane is thicker. × 800.

Figs. 141–143.—The early, mid, and late phases of probably the fourth nucleolar discharge; the granules in the cytoplasm are increasing in size and in number, and the membrane of the sporangia is becoming thicker; in fig. 143 the beginning of the deposition of the host cytoplasm upon the sporangium can be seen; × 800. Fig. 143*a* shows the differentiation of structure now visible in the granules of the cytoplasm. × 1650.

Fig. 144.—The granules of the cytoplasm at a later stage than in fig. 143*a*; all now show differentiation of structure; some of the larger are multiplying by constriction. × 1650.

Fig. 145.—The chromatin of the zoospore-primordia (*i.e.*, of the enlarged granules in the cytoplasm) has greatly increased in amount. × 1650.

Fig. 146.—Accumulation of chromatin at certain points of the zoospore-primordia causing them to become irregular. × 1650.

Fig. 147.—The giving off of the substance of the chromatin projections from the zoospore-primordia. × 1650.

Fig. 147*a*.—Many granules are being given off, and those already emitted lie between the primordia. × 1650.

Fig. 147*b*.—The granules which were given off have ceased to stain deeply and have almost disappeared; occasional granules are still being emitted; the chromatin of the primordia is much reduced in quantity. × 1650.

Fig. 148.—A few colourless irregular structures on the strands of cytoplasm are all that remain of the emitted granules. × 1650.

Fig. 149.—The few small chromatin granules of the primordia are being replaced by single larger ones. × 1650.

Plate 16.

Fig. 150.—Stages in the constriction of the large primordia of which there are usually several in each sporangium.

Fig. 150*a*.—The difference in size between a normal and a large zoospore-primordium is shown. × 1650.

Fig. 151.—A zoospore beginning to expand as the result of absorption of water; the single chromatin granule has enlarged. × 1650.

Fig. 152.—The single chromatin granule forming the nucleus has given rise to several granules and a clear area has developed in their midst; the cytoplasm of the zoospore is much vacuolated. × 1650.

Fig. 153.—Mature zoospore with blepharoplast and the deeply-staining strand which connects it with the nucleus; the cytoplasm is now arranged in delicate strands round the periphery of the zoospore while the centre is occupied by a large vacuole. × 1650.

The Appearance of the Original Nucleus of the Resting Sporangium during the Later Stages of Development of the Zoospores.

Fig. 154.—The distribution of the linin matrix resulting from a nucleolar discharge, probably following the stage shown in the sporangium of fig. 143; wide spaces are present in the reticulum; the nucleolus is vacuolated but still budding. × 800.

Fig. 155.—The distribution of linin has continued and the width of the mesh has increased. × 800.

The Formation of the Outermost Layer of the Wall of the Resting Sporangium.

Fig. 156.—A portion of the sporangium and host cell before the formation of the epispore (outermost layer); disintegration products of an oily nature have been formed in the protoplasm on the sporangium and in that on the host walls; unbleached material. × 1650.

Fig. 157.—The vacuole region of the host cell has a milk white gelatinous appearance as its contents change to form the membrane; the disintegration globules are conspicuous on the sporangium; unbleached material. × 1650.

Fig. 158.—The membrane after it has become yellow in colour; the disintegration globules will remain at the inner and outer surfaces of the newly formed layer; unbleached material. × 1650.

The Division of the Infected Host Cell.

Fig. 159.—A small portion of the epidermal region of the host tissue when the widespread first division is taking place; two walls have been formed parallel to the surface, and one is oblique; the parasites lie in the lower ends of the host cells. × 400.

Fig. 160.—Four adjacent epidermal cells, each containing several parasites; division of the host cells has not yet occurred; all the parasites are smaller than the host nuclei. × 780.

Figs. 161–163.—Prophase, metaphase and telophase respectively of the division of the epidermal host cell; in every case the parasites will be cut off in the inner daughter cell. × 780.

Fig. 164.—Division of a sub-epidermal cell; the second wall will be set obliquely to the first division wall, and the parasite will then lie in the innermost cell. × 780.

Fig. 165.—Division (prophase) of a deeply placed host cell; the sporangium has already formed a thick membrane. × 400.

Figs. 166, *a*, *b*.—Six parasites contained in adjacent sections of two daughter cells; the parasites have been separated into two groups on the division of the original cell, and would have been further distributed by the repeated division of both daughter host cells. × 780.

Discharge of the Zoospores of the Resting Sporangia.

Fig. 167.—The innermost layer of the membrane of the sporangium has become strikingly hyaline; the sporangium is distended and the zoospores, which are not shown in this figure, are separating from one another. × 780.

Fig. 168.—The conical projection, derived from the hyaline innermost layer of the membrane, the formation of which causes the sporangium to burst. × 780.

Fig. 169.—Ruptured empty sporangium; the hyaline layer is swollen and lies in folds within the two outer layers of the membrane. × 780.

Fig. 170.—Surface view of a living sporangium during the maturation of the zoospore-primordia. × 780.

Structure and Entry of the Zoospore.

Fig. 171.—View of living zoospore; the anterior refractive spot marks the position of the nucleus. × 780.

Fig. 172.—Zoospore, fixed and stained, showing nucleus and blepharoplast, and the deeply staining strand connecting the two. × 780.

Fig. 173.—Zoospores in a cleft on the surface of the host plant; cilium-reduction can be seen in several cases. × 800.

Fig. 174.—Zoospore at rest on the host surface before entry begins; nucleus, blepharoplast, and connecting strand are all visible. × 2000.

Figs. 175–177.—Stages in the elongation of the nuclear projection towards the surface of contact. × 2000.

Figs. 178, *a–d*.—Stages in the formation of the nuclear projection when the nucleus lies close to the surface of contact and elongation is thus relatively slight. × 2000.

Fig. 179.—The tip of the nuclear projection is pressed against the surface of the zoospore immediately before entry begins. × 2000.

Fig. 180.—The contraction of the cilium; the nuclear projection reaches half way to the surface. × 2000.

Fig. 181.—Entry into the host cell of the apical region of the nuclear projection. × 2000.

Fig. 182.—A small quantity of cytoplasm has passed into the host cell; plasmolysis has occurred. × 2000.

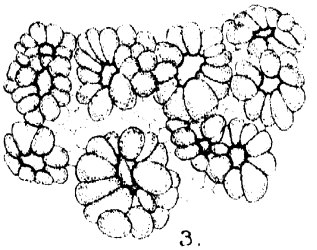
Fig. 183.—In both examples the nucleus has almost completed its entry and only a small amount of cytoplasm remains outside. × 2000.

Fig. 184.—Entry completed; the nucleus still extends towards the point of entry. × 2000.

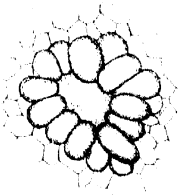
Fig. 185.—A later stage than that shown in fig. 184; the nuclear extensions have now been withdrawn. × 2000.



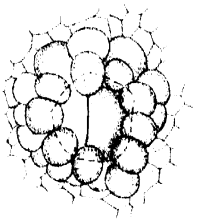
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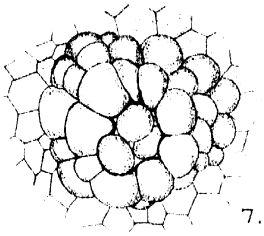
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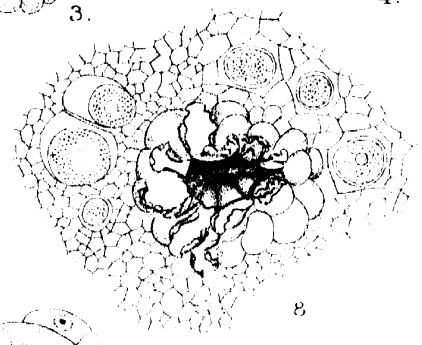
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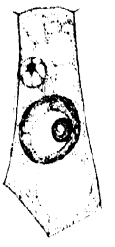
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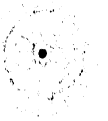
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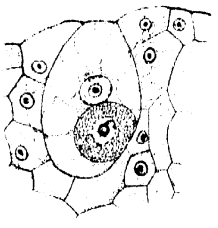
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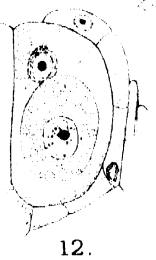
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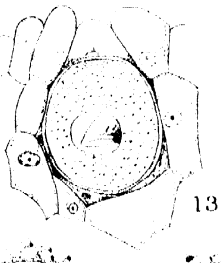
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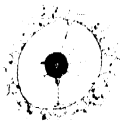
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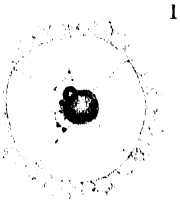
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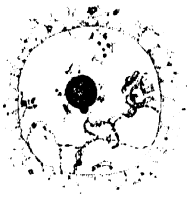
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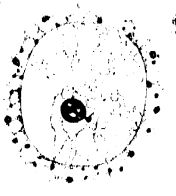
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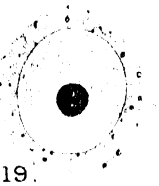


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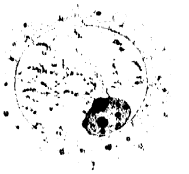




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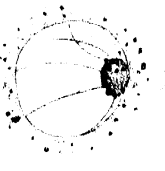
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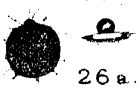
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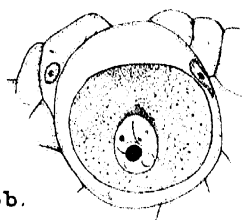
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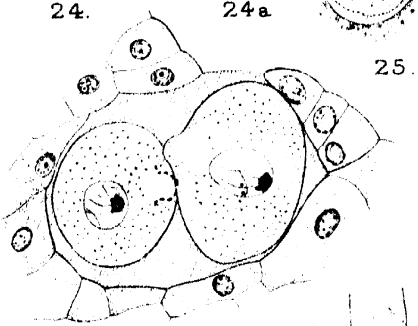
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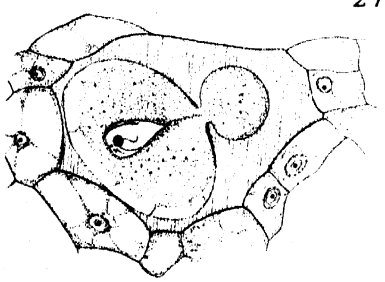
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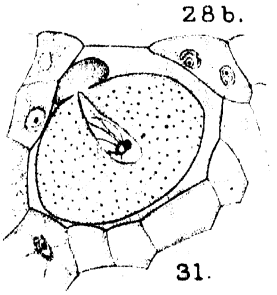
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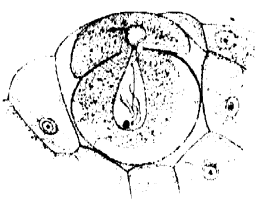
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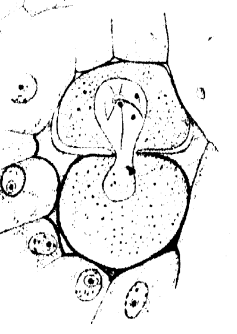
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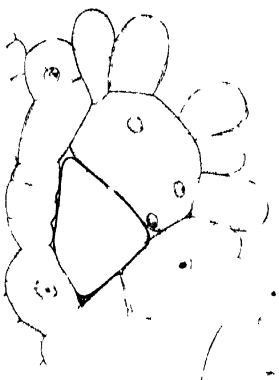
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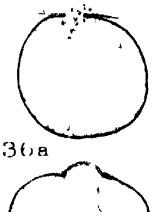
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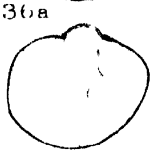
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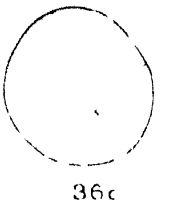
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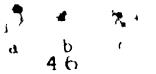
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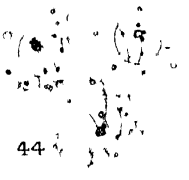
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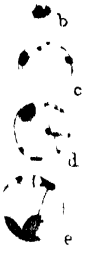
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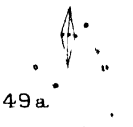
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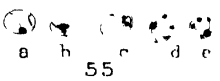
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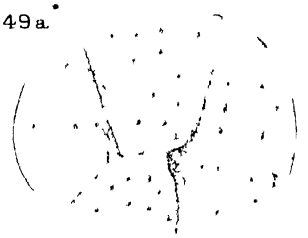
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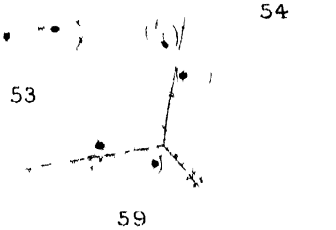
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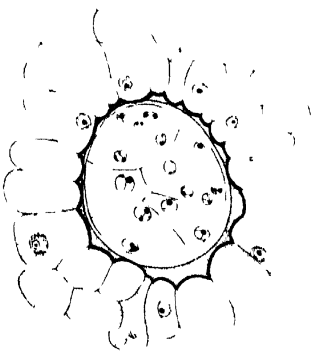


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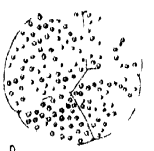
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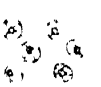
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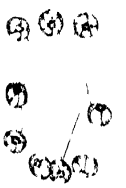
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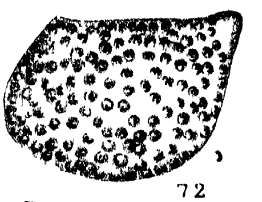
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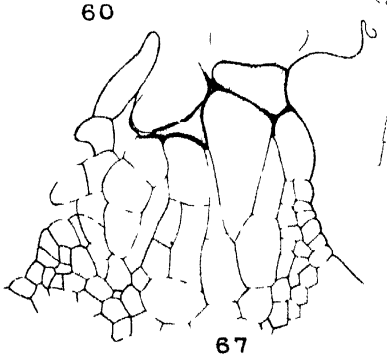
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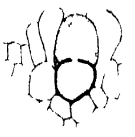
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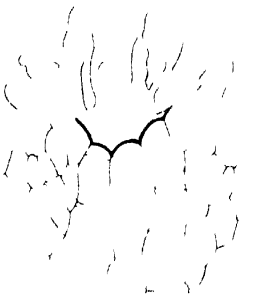
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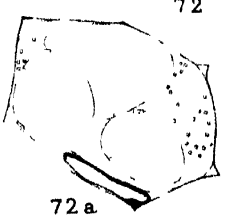
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—— Part IV...	1	2	6
1827. Part I...	0	18	0
—— Part II...	0	18	0
1828. Part I...	1	1	0
—— Part II...	0	10	0
1829. Part I...	0	16	0
—— Part II...	0	14	0
1830. Part I...	1	10	0
—— Part II...	1	1	0
1831. Part I...	1	10	0
—— Part II...	1	12	0
1832. Part I...	1	1	0
—— Part II...	2	0	0
1833. Part I...	1	1	0
—— Part II....	2	18	0
1834. Part I...	0	17	0
—— Part II...	2	2	0

	£	s.	d.
1835. Part I...	1	2	0
—— Part II...	0	14	0
1836. Part I...	1	10	0
—— Part II...	2	0	0
1837. Part I...	1	8	0
—— Part II ..	1	8	0
1838. Part I...	0	13	0
— Part II...	1	8	0
1839. Part I...	0	18	0
- - Part II ..	1	1	6
1840. Part I ..	0	18	0
—— Part II...	2	5	0
1841 Part I ..	0	10	0
—— Part II ..	1	10	0
1842. Part I ..	0	16	0
—— Part II...	1	2	0
1843. Part I...	0	10	0
—— Part II...	1	10	0
1844 Part I...	0	10	0
- Part II...	1	10	0
1845 Part I...	0	16	0
—— Part II ..	1	0	0
1846 Part I...	0	7	6
—— Part II ..	1	12	0
—— Part III...	1	12	0
— Part IV...	1	12	0
1847. Part I...	0	14	0
—— Part II...	0	16	0
1848. Part I. .	1	0	0
—— Part II...	0	14	0
1849. Part I...	1	0	0
—— Part II...	2	5	0
1850. Part I...	1	10	0
—— Part II...	3	5	0
1851. Part I...	2	10	0
—— Part II...	2	10	0
1852. Part I...	1	0	0
—— Part II...	2	5	0
1853. Part I...	0	18	0
—— Part II...	0	12	0
—— Part III...	1	2	0
1854. Part I...	0	12	0
—— Part II...	0	16	0
1855. Part I...	0	16	0
—— Part II...	1	6	0
1856. Part I...	2	0	0
—— Part II...	1	4	0
—— Part III...	1	4	0
1857. Part I...	1	8	0
—— Part II...	1	4	0
—— Part III...	1	2	0
1858. Part I...	1	8	0
—— Part II...	3	0	0
1859. Part I...	2	10	0
—— Part II...	2	5	0
1860. Part I...	0	16	0
—— Part II...	2	1	6
1861. Part I...	1	3	0
—— Part II...	1	5	0
—— Part III...	1	7	6
1862. Part I...	2	14	0
—— Part II...	3	0	0

	£	s.	d.
1863. Part I...	1	14	0
—— Part II...	1	7	6
1864. Part I...	0	11	0
—— Part II...	1	7	6
—— Part III...	1	10	0
1865. Part I...	2	2	0
—— Part II...	1	5	0
1866 Part I...	1	14	0
—— Part II...	2	7	6
1867. Part I...	1	3	0
—— Part II...	1	15	0
1868. Part I...	2	5	0
—— Part II .	2	0	0
1869. Part I...	2	10	0
—— Part II...	3	3	0
1870. Part I...	1	10	0
—— Part II...	1	18	0
1871 Part I...	1	10	0
—— Part II...	2	5	0
1872. Part I...	1	12	0
—— Part II ..	2	8	0
1873. Part I...	2	10	0
—— Part II ..	1	5	0
1874. Part I...	2	8	0
—— Part II...	3	0	0
1875. Part I...	3	0	0
—— Part II...	3	0	0
1876. Part I...	2	8	0
—— Part II...	2	8	0
1877. Part I...	1	16	0
—— Part II...	2	5	0
Vol. 168 (extra)	3	0	0
1878. Part I...	1	16	0
—— Part II...	3	0	0
1879. Part I...	2	0	0
—— Part II...	1	12	0
1880. Part I...	2	5	0
—— Part II...	2	0	0
—— Part III...	1	1	0
1881. Part I...	2	10	0
—— Part II...	1	10	0
—— Part III...	2	2	0
1882 Part I...	1	14	0
—— Part II...	2	0	0
—— Part III...	2	10	0
—— Part IV...	1	0	0
1883. Part I...	1	10	0
—— Part II...	2	10	0
—— Part III...	1	12	0
1884. Part I...	1	8	0
—— Part II.	1	16	0
1885. Part I...	2	10	0
—— Part II...	2	5	0
1886. Part I...	1	8	0
—— Part II...	1	15	0
1887. (A.)	1	3	0
—— (B.)	1	16	0
1888. (A.)	1	10	0
—— (B.)	2	17	6
1889. (A.)	1	18	0
—— (B.)	1	14	0
1890. (A.)	1	16	6
—— (B.)	1	5	0
1891. (A.)	2	2	0
—— (B.)	3	3	0
1892. (A.)	2	1	0
—— (B.)	2	2	0

	£	s.	d
1893. (A.)	3	14	0
—— (B.)	2	13	0
1894. (A.) Pt. I.	1	5	0
—— (A.) Pt. II.	1	10	0
— (B.) Pt. I.	3	10	0
—— (B.) Pt. II.	1	11	6
1895. (A.) Pt. I.	1	14	0
—— (A.) Pt. II.	1	3	6
(B.) Pt. I.	1	15	0
—— (B.) Pt. II.	0	16	6
1896 (A) Vol. 187.	1	19	6
—— (A) Vol. 188.	1	9	0
—— (B) Vol. 187.	0	19	0
1897 (B) Vol. 188.	0	19	6
—— (A) Vol. 189.	1	0	0
—— (A) Vol. 190.	1	8	6
-- (B) Vol. 189.	0	17	6
1898 (B) Vol. 190.	2	5	0
—— (A) Vol. 191.	1	16	0
1899 (B) Vol. 191.	1	12	0
—— (A) Vol. 192.	1	8	6
1900 (B) Vol. 192.	1	7	6
— (A) Vol. 193.	1	10	0
- (A) Vol. 194.	1	1	6
— — (B) Vol. 193.	1	5	0
1901 (A) Vol. 195.	1	7	0
—— (A) Vol. 196.	1	11	0
— (B) Vol. 194.	3	0	0
—— (A) Vol. 197.	1	15	0
1902 (A) Vol. 198.	1	8	6
—— (A) Vol. 199.	1	6	0
1903 (A) Vol. 200.	2	3	0
—— (A) Vol. 201.	1	16	6
—— (B) Vol. 195.	1	7	0
1904 (A) Vol. 202.	1	13	0
—— (A) Vol. 203.	1	1	6
—— (B) Vol. 196.	1	16	0
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—— (B) Vol. 197.	1	13	6
1906 (A) Vol. 205.	1	8	0
—— (A) Vol. 206.	1	8	6
—— (B) Vol. 198.	2	1	0
1908 (A) Vol. 207.	1	13	6
—— (A) Vol. 208.	1	18	6
—— (B) Vol. 199.	1	16	0
1909 (A) Vol. 209.	1	13	0
—— (B) Vol. 200.	2	5	6
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—— (B) Vol. 201.	1	15	6
1912 (A) Vol. 211.	1	6	6
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1914 (A) Vol. 213.	1	6	0
—— (A) Vol. 214.	1	11	6
—— (B) Vol. 204.	1	12	6
—— (B) Vol. 205.	2	3	6
1915 (A) Vol. 215.	1	11	0
—— (B) Vol. 206.	1	15	0
1916 (A) Vol. 216.	1	10	0
—— (B) Vol. 207.	2	8	6
1918 (A) Vol. 217.	1	11	0
—— (B) Vol. 208.	2	7	0
1919 (A) Vol. 218.	1	15	0
—— (A) Vol. 219.	0	17	6
1920 (B) Vol. 209.	3	13	0
—— (A) Vol. 220.	2	4	0
1921 (B) Vol. 210.	3	5	6

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