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LECTURES ON BIOCHEMISTRY

LECTURES ON
CERTAIN ASPECTS OF BIOCHEMISTRY

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THESE LECTURES WERE GIVEN
IN THE UNIVERSITY OF LONDON
DURING THE SUMMER TERM, 1925

LONDON
UNIVERSITY OF LONDON PRESS, LTD
17 WARWICK SQUARE, E.C.4
1926

Printed in Great Britain for the UNIVERSITY OF LONDON PRESS, LTD.,
by HAZELL, WATSON AND VINEY, LD., London and Aylesbury.

PREFACE

In the Summer Term, 1925, three series of lectures were delivered in the University of London, to advanced students and others, on subjects coming most naturally under the heading of Biochemistry. One series was given at University College by two Professors of the University, the other two by Dr. H. H. Dale, of the National Institute for Medical Research, and Professor L. J. Henderson, of Harvard. The coincidence of so many lectures on different problems of Biochemistry prompted us to put them together in a volume. The lectures have been printed in the form in which they were delivered, except where the use of diagrams and lantern slides, not here reproduced, necessitated minor modifications of phraseology. No attempt has been made to give them the finality of a text-book, or the completeness of monographs. They represent only certain aspects of Physiology which have appealed to us, and our approach to these has, in general, been from the side of Biochemistry, though perhaps some of the matters considered might not usually have been classed under that heading.

A. V. Hill.

University College,
London
December, 1925.

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LECTURE I

THE CONTROL OF THE CIRCULATION IN THE CAPILLARY BLOOD-VESSELS

UNTIL quite recent years it was the generally accepted view that the rôle of the capillaries, so far as the mechanical aspect of the circulation was concerned, was a purely passive one. They were conceived as a network of lax, membranous tubes of endothelium, admirably adapted to their known function of permitting the diffusional interchange between the blood and the tissues, needed for the nutritive and respiratory functions of the blood, and to the processes of ultra-filtration concerned in the formation of the lymph and the urine. The available path for the blood provided by the capillary network, as seen in injected specimens, appeared to be so large, that it seemed impossible to suppose that the blood, flowing through it in a slow stream, encountered any serious frictional resistance. Attempts were made to give a quantitative precision to this view by measuring the average rate of flow, through such capillaries as could be observed in the living condition under the microscope, and comparing this with the rate of flow in the aorta. The result was to show that the blood was moving one or two thousand times as fast in the aorta as in the capillaries; and

accordingly the total sectional area of the capillary path was calculated to be about one or two thousand times as great as that of the aorta.

On the assumptions that the capillaries are simply lax, membranous tubes, passively filling and collapsing as the arteries supplying them open or close down, and that they allow at all times free passage to the blood and its corpuscles, such a conclusion would be perfectly justified ; and the corollary that, flowing so slowly through such a wide path, the blood would encounter hardly a significant constituent of the peripheral resistance, would be obvious and inevitable. Along such lines of reasoning we arrive at the long-familiar scheme of distribution of pressure in the systemic circulation, in which the pressure is represented as falling very slowly as the blood passes from the heart along the larger arteries, falling with ever-increasing steepness as these branch into the smaller arteries and arterioles, attaining a low level and again suddenly acquiring a gentle gradient where the arterioles open into the capillaries. On such a scheme, if the output of the heart remained constant, the pressure in the larger arteries, on the one side, and the rate of flow in the capillaries on the other, would be determined and varied almost entirely by the changing tone in the small arteries, where the blood would encounter practically the whole, and the only variable constituent, of the peripheral resistance to its flow. The changing tone of the arterioles is, on this view, solely responsible for the regulation of the blood-supply as between different organs and

tissues, in accordance with their varying metabolic needs.

You may have noticed, however, that I mentioned two assumptions, as involved in the deduction of such a scheme from the available evidence.

(1) The first was that the capillaries have no power of actively varying their lumen. If we admit them to possess contractility, like that of the small arteries, it is obvious that we cannot safely calculate the total capillary path available at any one moment, either by looking at artificially injected specimens, in which all the capillaries are dead and distended, or by looking at samples under the microscope of such living capillaries as can thus easily be seen, and assuming that the observed relation between their total bore and that of the artery supplying them, and consequently the rate of flow in them, prevails throughout all the tissues of the body.

(2) The second assumption was that the corpuscles would always have such free passage through the vessels that, without significant error, the blood might be regarded as a homogeneous fluid, having such moderate viscosity as could be determined by an ordinary viscosimeter. If once we have evidence, however, that the capillaries are independently contractile, and that contractile tone is normally so effective in them, that a large proportion are closed altogether, and a further large proportion so contracted that the red corpuscles cannot pass through them without elastic deformation, the situation is completely altered.

Now it is in these directions that the evidence of recent years has necessitated, as it seems to me, a serious revision of our conception of the function of the capillaries, in relation to the mechanics of the circulation.

Older evidence was not wanting in favour of an independent contractility of the capillaries. They had been seen to contract and expand automatically, even in excised tissues, in which there was no arterial pressure to produce passive changes. Rouget described contractile cells, embracing the endothelial tubes of the capillaries with slender processes, and thirty years later Mayer confirmed his description, and maintained that this layer of cells was continuous, through intermediate forms, with the plain muscle coats of the arterioles and of the venules. Steinach and Kahn described the contraction of these cells as pleating and folding the endothelial capillary tube, till its lumen might be obliterated. Langley had suggested that the ischæmia, produced by locally applying adrenaline to a mucous membrane, was too complete to be attributed to arterial contraction alone.

But while it had to be admitted, on such evidence, that the capillaries might have some independent power of contraction, especially in embryonic and amphibian tissues, on which most of the observations had been made, nobody seemed prepared to suppose that this function had any importance for the control and maintenance of the circulation under normal conditions. Langley had only gone so far as to

suggest that it might have some regulating influence when the arterial pressure was very low.

It was not till about 1917 that the contractile function of the capillaries began to come into serious notice, as having real physiological importance; and then the evidence began to appear from several different quarters, in which simultaneous and independent observations had been proceeding. The first of these newer publications was made by Cotton, Slade, and Lewis, who investigated the so-called dermographic phenomena, and showed that, of the local vasomotor reactions of the human skin to mild mechanical stimulation, those known as the red and the white "tache" could be produced perfectly well with the circulation arrested by closing the arteries, so that they must be attributed to active relaxation and closure, respectively, of the capillaries in the skin. The diffuse pink flush, surrounding these more localised reactions, was obliterated by closing the arteries, and was obviously due to reflex arterial dilatation.

Cotton, Slade, and Lewis also found that adrenaline, injected into the skin, would cause a spreading patch of pallor even when the circulation was stopped, showing the correctness of Langley's assumption that adrenaline would directly stimulate the capillaries to contraction. Ebbecke, in the same year, published a long memoir, covering much of the same ground, and added the important point that a line of local capillary dilatation is produced, not only in the skin, but on the surface of an internal organ, by stroking

pressure. Both sets of observers noticed that, if the capillaries were thus stimulated to relaxation beyond a certain point, they became incapable of retaining the plasma, and an œdematous weal resulted. In some subjects this reaction is very readily produced, causing the so-called "factitious urticaria."

In the next year Krogh published the first of the now well-known series of papers on this subject, which have issued from his school. He started from an entirely different point of view. He was impressed by the enormous disparity between the oxygen needed and used by a muscle, in activity and at rest respectively, and by the extent to which, in order to meet that need, the blood supply must be increased, and the blood brought into closer relation to the oxygen-using fibres, when the muscle became active. He came to the conclusion that this could only be effected by a great increase in the number of capillaries open and transmitting blood. By an ingenious method of intravital injection with Indian ink, he was able to count and measure the capillaries through which blood was passing, in a muscle under these different conditions. One count showed 85 capillaries open in a certain unit of cross-sectional area of the muscle at rest, as compared with 2,500 open in the same area during activity—a ratio of 1:30. The contrast in the average diameter of those which were open under the two conditions was similarly large, and Krogh estimated the volume of blood, contained at one moment in a muscle, as varying from $\frac{1}{5000}$ of the muscle volume at rest, to $\frac{1}{15}$ of the muscle

volume during activity, and $\frac{1}{8}$ to $\frac{1}{4}$ of the muscle volume if the whole available capillary network was fully dilated. A simple calculation will show that, if we allow the bulk of the muscles to be about 40 per cent of that of the whole body, the fully dilated capillaries of the muscles alone could hold a quantity of blood amounting to about $\frac{1}{15}$ of the body weight—i.e. the whole of the blood in the body.

Using his Indian ink method Krogh was able, further, to observe the flow of blood in the capillaries, and the relation of their diameters to that of the red corpuscles, in the living muscle. In the capillaries thus seen under the microscope in the living and resting muscle, and drawn by Krogh, the most widely open will only just let the red corpuscles pass without deformation, while in the others varying degrees of distortion and elongation are needed to allow the elastic corpuscles to be squeezed through. It must further be borne in mind that the capillaries which are open, and therefore visible at all by the method employed, are a small minority. Some figures of Krogh's actual measurements of the diameters of capillaries will make the point clear. In a series of capillaries in the resting muscle of the guinea-pig's abdominal wall the following were the measured diameters of the lumina in μ: 2·2, 4·1, 3·0, 4·2, 4·5, 2·7, 3·7, 2·5, 2·8, 4·0, 1·8, 3·8, 3·0, 3·5, 6·5, 3·1, 2·9, 5·0, 3·3. The *average* diameter is 3·5 μ, and probably only one of the capillaries measured (6·5 μ) would have allowed the guinea-pig's red corpuscles to pass without sensible deformation.

I think it can hardly be doubted that, in passing through such vessels, the blood will encounter a very serious part of the peripheral resistance to its flow, or that a sudden widening of such capillaries, so as to allow the corpuscles to pass freely, and the simultaneous opening of a much larger number, which were previously closed altogether, will cause a sudden weakening of the peripheral resistance. If such a capillary dilatation took place simultaneously in a large part of the body, it must result in a fall of the pressure in the large arteries, which we commonly call the " arterial pressure."

We have spoken thus far of the capillaries in the skeletal muscles—the most abundant of all the tissues. Krogh states that quite similar conditions are to be observed in the capillaries of the walls of the alimentary canal; and these, with those of the skeletal muscles, must together account for a very large part of the capillary network of the body, and have a large effect on the total peripheral resistance. We have no reason, on the other hand, to suppose that similar conditions obtain in the capillaries of such an organ as the lung or the liver. Krogh and his co-workers have entirely confirmed and added further detail to Rouget's description of the contractile cells surrounding the endothelial tube of the capillary, and they make it clear that the line of anatomical and physiological distinction, between capillaries and arterioles on the one hand, or capillaries and venules on the other, is much less sharp than used to be supposed. They have further, by direct

observation, reached the important conclusion, already implicit in the dermographic reactions, that the permeability of a capillary wall increases with its relaxation, until, when fully relaxed, it can no longer retain the blood plasma.

Passing now to another of the independent investigations on capillary function, which were in progress during the early years of the war, we come to one aspect of what is the main theme of my lecture to-day, in that we deal with one kind of chemical influence modifying the circulation in the capillaries. My colleagues and I had been working at intervals for some years on the action of the substance histamine, an organic base which is produced from most proteins by putrefaction, being formed by decarboxylation from the amino-acid histidine. We were interested in it, not so much for its own sake, as because its action, in several directions, resembled and typified that of a large group of cleavage products, formed from proteins by enzymes or chemical agents. This substance when injected intravenously, even in minute doses, produced a fall of arterial blood-pressure, very closely similar to that caused by a typical arterial dilator.

On the other hand, while the nitrites and the choline esters could be shown to produce dilatation even of isolated arteries, nobody had ever been able thus to demonstrate that histamine did anything but constrict them. Further, unlike the arterial dilators, histamine in larger doses caused a profound and irremediable collapse of the circulation. This was shown to be due to the passage of a large part of

the blood out of the currency of the circulation, so that it remained somewhere at the periphery and did not return to the heart. Further, as this condition developed, part of the plasma passed out of the vessels, so that the blood remaining in circulation became thick with excess of corpuscles.

A number of different lines of evidence, which it would take me too long to put before you to-day, brought us to the conclusion that histamine produced all these effects because its dilator action was exerted, not on the arteries, but chiefly, if not entirely, on the capillaries. To justify that conclusion we had to make a series of assumptions, for which, at the time, there appeared to be no warrant. We assumed that the capillaries not only had an independent contractility, but that this was normally in such effective action that a large part of the capillary network was closed, and that only a small proportion of the available channels was at any one moment open and transmitting blood. We had to assume that, under such conditions, a material part of the peripheral resistance to the blood flow was encountered in the capillaries, and that a sudden relaxation, and opening up of fresh channels, would weaken this resistance and consequently lower the arterial pressure. We had to assume that if all the capillary network were simultaneously and widely relaxed, its capacity would become such that a large part of the blood would be accommodated in it, and, there lying stagnant, fail to return to the heart. Lastly, we had to assume that, when the capillary

walls were thus relaxed, they became so permeable that the plasma passed out through them, leaving the blood thick with corpuscles. All these assumptions, which at the time seemed highly unorthodox, received direct evidence in their support from the work of Krogh and his co-workers, which I have already mentioned, and the earliest part of which was in the press at the same time as our own. I think it may be regarded as very likely, therefore, that they were, in the main, correct.

There was one observation, in particular, made by Prof. Richards and myself, for which we found it very difficult to give an adequate explanation. It was one of the anomalies in connection with histamine, that nobody had then succeeded in demonstrating its vasodilator action in an organ artificially perfused. When perfusion was carried out with a saline solution, with gum to give it viscosity, the tone of the vessels could be put up with adrenaline till quite a high pressure was needed to force the saline solution through them at a reasonable rate, but histamine, if it did anything, would still only make the arteries constrict further. Richards and I found that if we used blood for perfusion, or even added washed corpuscles to the saline solution, and then put up a tone in the vessels with a little adrenaline, we obtained a perfectly typical vasodilatation on injecting histamine. I believe that the significance of the corpuscles probably finds its explanation also in Krogh's observations. You will remember his description of the corpuscles just squeezing through the narrowed

capillaries, and my suggestion that, with corpuscles present and the capillaries in tone, an important part of the peripheral resistance must be in the capillaries, so that their relaxation would produce all the phenomena we associate with peripheral vasodilatation. In the absence of corpuscles, however, it may well be that, though the individual capillaries are narrow, the total path they provide may be so wide in relation to that in the arterioles, that their relaxation has very little apparent effect on the resistance to flow, and that the only effect we then observe, when we inject histamine, is that of arterial constriction.

One other point I should like to make a little clearer. I have never supposed that the change of reaction to histamine necessarily occurs exactly at the line of distinction between what we should recognise histologically as arterioles and capillaries respectively. Krogh's description forbids us to regard the change either of structure or function as abrupt. Our evidence seems to indicate that the change of reaction to histamine, from contraction to relaxation, takes place in the cat at a level more peripheral than that of the smallest arteries we can separate anatomically; but the still finer arteries may be involved with the true capillaries in the dilator action. This seems the more likely in that Dr. Burn and I have quite recently found that in the dog the dilator effect of histamine spreads further back, and involves even arteries which can be separately recognised as such.

There is nothing, however, to disturb our conclusion that the main incidence of the vasodilator effect of histamine is on the capillaries, and most of the more recent evidence has tended to reinforce that conclusion. And the same applies to the action of the less chemically definite products of protein cleavage, such as peptones, albumoses, etc.

So far, then, we have evidence before us which seems to justify the conclusion, that capillary contractility and capillary tone are not merely curious phenomena to be observed in the vessels of embryos or of cold-blooded animals, but functions of real and general importance for the maintenance and regulation of the circulation. There is good reason to believe that a general collapse of the capillary tone, with resulting stagnation of a large part of the blood in the tissues, such as can be experimentally produced by histamine or peptone, is a phenomenon of real pathological significance, occurring, for example, in the condition which was called "secondary wound shock" during the war, or in the terminal circulatory collapse attending a rapidly spreading or generalised bacterial infection. My purpose to-day, however, is to discuss with you what bearing these observations may have on the physiology of the normal circulation. Capillary tone being recognised as an essential factor in an efficient circulation of the blood, we have to enquire what means the body possesses for maintaining it and varying it within physiological limits.

In the first place, there is good evidence that the tone of the capillaries, like that of the arterioles, is

under control of the nervous system. Steinach and Kahn first showed that stimulation of sympathetic nerves made the capillaries as well as the arterioles contract, and their evidence has been confirmed and extended by Krogh. Since artificial stimulation of these nerves makes the capillaries contract, it is reasonable to suppose that through them the capillaries, like the arteries, are kept in tone by action of the vasomotor centres in the bulb and spinal cord, though direct evidence on the point would be very difficult to obtain.

Then you will be familiar with the phenomenon of the so-called antidromic vasodilatation, discovered by Stricker, and demonstrated in a much more convincing form by Bayliss, who studied it in great detail. When the dorsal spinal roots are cut, and the peripheral ends artificially stimulated, vasodilatation is produced in the area supplied by them. So far as is known, these roots consist entirely of fibres of which the normal function is sensory. The view commonly held is that a sensory fibre, near its termination in a sensory ending, sends off a side-branch which ends in relation to the small blood-vessels. A probable function of this curious distribution is the production of vasodilatation by local reflex-action through the axon branching, in response to irritation of sensory nerve-endings in the neighbourhood of the vessels affected. There is good evidence that the capillaries, as well as the arterioles, receive this type of innervation.

But, as in the case of the arterioles again, the body

has other than nervous mechanisms at its disposal for maintaining and changing the tone of the capillaries. I have said much already concerning one kind of chemical stimulus, by which relaxation of the capillaries is produced ; and it will be convenient at this point to consider its possible function under normal conditions.

We have already spoken of its connection with the effects of massive injury. Is there any reason to suppose that a similar chemical agent of capillary relaxation is concerned in the fleeting effects of trivial injury, such as the red " tache " formed when the skin is subjected to stroking pressure ? Lewis and his colleagues, in the last year, have collected a large amount of interesting evidence which is at least suggestive in that direction. Sollmann and Pilcher had shown that, if histamine in high dilution is injected, or simply pricked into the skin, a local capillary dilatation is produced, followed by wheal formation. Lewis and Grant have shown that the conditions favouring, depressing, or preventing this reaction to histamine are exactly those which favour, depress, or prevent the formation of the red line and the wheal in response to local mechanical stimulation. The evidence strongly suggests, though it does not prove, that something having what, for want of a better description, we may call the " histamine " type of action, is formed in the tissues subjected to this very mild trauma.

When we further enquire whether substances acting thus are formed in normal metabolism, the evidence

is again suggestive, though again not conclusive. It is suggestive that a simple extract, made by boiling the fresh tissue of almost any organ in the body, will be found to contain some substance or substances, quite unidentified, which have this histamine-like action. Either such substances are pre-formed in the tissues during life, or formed from them very readily in the act of death. Then we have the interesting phenomena which follow temporary stoppage of the arterial blood-supply to a tissue. When the blood is readmitted, the capillaries are found to be widely dilated, and remain in this condition for so long afterwards, that it is hardly credible that the temporary lack of oxygen or accumulation of CO_2 could account for it. The phenomenon was mentioned in 1879 by Roy and Graham Brown, and has recently been extensively investigated by Lewis and his colleagues. Its features strongly suggest the accumulation in the tissues, deprived for the time of the washing effect of the blood, of something which, again, has this histamine-like action.

Now, if we suppose that the tissues, even at rest, are normally producing something which thus relaxes the capillaries, the question naturally arises, whether the production of such an agent is accelerated during active metabolism. Unfortunately the possibility is quite beyond the reach of test by the chemical means available. Histamine itself will, on occasion, produce a measurable fall of arterial pressure, due to general capillary relaxation, when injected into the circulation of a cat in a dose of $\frac{1}{100,000,000}$ of a milligram. The

amount of such a substance which would have to be produced locally, outside the blood current, in order to produce a local relaxation of capillaries, is altogether beyond the range of any yet conceivable means of chemical detection. We must be content, for the present, to speak teleologically, and to say that, if a substance having this kind of action were formed in metabolism, and if its production were accelerated in proportion to the intensity of metabolism, it would be an ideal agent for the fine adjustment of the circulation to the needs not only of an organ, or part of an organ, but even of an individual cell. Some mechanism, other than the known nervous mechanisms, has long been recognised as necessary, to explain the extraordinary increase of blood-supply which accompanies activity in a muscle, a gland, or other intermittently active organ. The tendency of all recent evidence is to suggest that, in this increase, relaxation of the capillaries plays even a more important part than that of the arterioles; and it is obvious that, in so far as the effect is produced by any kind of chemical agent formed by the active tissues, the capillaries, with their intimate physiological contact with those tissues, are in a peculiarly favourable position to be influenced by that agent.

So far we have dealt with possible chemical agents of capillary relaxation. With reference to the maintenance and restoration of normal capillary tone, we have hitherto spoken only of a probable influence of nervous centres. On the analogy of other functions we should expect that this nervous control would also

be supplemented by chemical stimulants. There is good evidence that it is. When the sympathetic nerve-supply, or indeed the whole nerve-supply, to a tissue is interrupted by section, the immediate effect is a wide relaxation of arteries and capillaries. A recovery of tone rapidly sets in, however, and this appears to be more rapid in the capillaries than in the arteries, so that even a few hours after section, when the arterioles are still widely relaxed, the capillaries may have acquired a tone greater than the normal, which may then be indefinitely maintained. Some chemical agents in the blood must be concerned, and, though there may be others of which we know nothing as yet, we do know with some definiteness of two.

I mentioned earlier the fact that addition of a small proportion of adrenaline, to the blood used for artificial perfusion of an organ, would set up a tone in the capillaries which histamine would then relax. I also mentioned the direct constrictor effect of adrenaline on capillaries, as observed by Langley, by Cotton, Slade, and Lewis, and by others. The question of most interest for us, however, is whether adrenaline, as ordinarily secreted into the blood by the suprarenal glands, plays any part in the maintenance of the normal capillary tone and in its restoration, when it has been relaxed, in the manner already considered. I think there is good evidence in favour of the view that it does. Several observers, myself among them, have observed that operative removal of the suprarenal glands from a cat is followed by a

gradual loss of plasma from the blood, so that by the time—usually after about forty-eight hours—that the terminal condition of collapse sets in, the blood has become very abnormally rich in corpuscles. Even before this stage the animal shows an extraordinary susceptibility to the injection of histamine into its circulation, so that a dose which produces no more than insignificant and fleeting symptoms in a normal cat, will produce a profound and even fatal collapse. My former colleague, Prof. Kellaway, made the point clearer by destroying the medulla only of the suprarenal glands of cats, leaving enough cortex to maintain the animal in relatively normal health. In such a cat, also, a dose of histamine which hardly affected a normal cat was found to produce a severe circulatory collapse.

Histamine and adrenaline, indeed, are strikingly antagonistic in their actions in a number of directions ; and it is again suggestive to find evidence that a small dose of histamine may excite, in a cat, a prompt response of the suprarenal gland, with accelerated output of adrenaline.

From several directions, therefore, we seem to have evidence of an effective physiological antagonism between this one of the endocrine functions of the suprarenal gland, and the chemical mechanism of capillary relaxation, of which we have evidence both in physiological and pathological conditions. It seems to me, indeed, to provide a clearer reason for the need of adrenaline to the maintenance of the normal circulation than we can find elsewhere.

The other bodily constituent known to possess, when artificially injected, a powerful vasoconstrictor action is an active principle which can be extracted from the posterior lobe of the pituitary body. As we shall be dealing with the principles contained in that organ in my second lecture, I will defer till then a discussion of the evidence as to its relation to the maintenance of normal capillary tone.

LITERATURE

Full references to most of the points considered in this lecture will be found in Prof. Krogh's book, *The Anatomy and Physiology of Capillaries*. Yale University Press. 1922. For Lewis's later work, see recent volumes of *Heart*.

LECTURE II

ACTIVE PRINCIPLES OF THE PITUITARY BODY

In my first lecture I endeavoured to put before you evidence in favour of the view that a maintenance of the capillary blood-vessels, as well as the arterioles, in a satisfactory average state of tone, is an essential condition of a normal and efficient circulation. We considered a class of substances by the action of which that tone is relaxed, and I began the consideration of normal chemical agencies, by which that tone can be maintained, restored, or increased, and dealt with the importance of adrenaline in that direction. I just mentioned that there is reason to suppose that the other known powerful vasoconstrictor substance produced by the body, that formed in the posterior lobe of the pituitary gland, has also some importance for the maintenance of the tone in the capillaries. The first direct evidence on the subject was produced by Krogh and his co-workers. Studying the capillaries in the skin of the frog's foot, they found that, if these were perfused with an ordinary Ringer's solution, they rapidly lost their normal tone, and, as they relaxed, became permeable, so that the fluid passed through their walls into the tissue spaces, and an œdematous condition resulted. This, as I explained in my first lecture, is a normal

result of the relaxation of tone in the capillaries. They proceeded to examine the constituents of normal blood by which the tone of the capillaries is upheld, and their retentive power for the blood fluid preserved. They found that addition of a proportion of ox serum, to the Ringer's solution perfusing the frog's leg, was sufficient to keep the capillaries in tone and prevent the œdema. The effect was not due to the colloidal proteins in the serum, since the substance producing it could be dialysed through a collodion membrane, and was stable to boiling. From these properties, and other points of chemical similarity, they were led to suspect that it might be an active principle from the pituitary gland. It cannot be suggested that the chemical evidence was strong, since the points of correspondence were not at all of a distinctive nature ; they belong to a very large number of substances. Somewhat more significant was a point of physiological similarity. When the frog's leg is perfused with Ringer's solution, as the capillary walls relax, the melanophores in the skin become contracted, so that the skin, whatever its initial appearance, becomes very pale. When serum is added to the perfusion fluid the melanophores expand again, and the skin becomes darker in colour. Now there is only one known active principle in the body which, in very high dilution, will produce this expansion of the melanophores, and that is an active principle derived from the pituitary body. I shall have occasion to mention this again later. For our present purpose

the important point is that Krogh and Rehberg found that a trace of pituitary extract would effectively replace serum in their perfusion fluid, and like this would prevent both the relaxation of the skin capillaries and the contraction of the melanophores. Further, and in confirmation of Pohle, they found that removal of the pituitary body from a frog, by operation, was followed, not only by pallor of its skin from contraction of melanophores, as Hogben and Winton had already observed, but also by the gradual development of cutaneous œdema, which they attributed to relaxation of the capillary vessels.

Krogh concludes that the hormone concerned with maintaining a healthy tone in the capillaries of the frog's skin comes from the pituitary body, and that the presence of traces of this hormone in the circulating blood is responsible for the effect of serum. He failed to find evidence, however, that it had a similar tonic effect on the capillaries in other organs of the frog, such as the muscles.

Several observers, among them Sacks, working in Lewis's department, have since noticed a very striking response of the capillaries of human skin to pituitary extract. If a small dose of the ordinary commercial extract of the pituitary posterior lobe is injected intravenously into a normal man, a death-like pallor is produced, which can only be attributed to strong contraction of the skin capillaries. It is not accompanied by more than a relatively trifling rise in the general arterial pressure, so that we must suppose that the action is restricted to the capillaries of the skin.

The subject suffers from no significant symptoms or definite discomfort. The meaning of this restriction to the skin vessels is not clear. It may be that the capillaries in other parts of the human body are insensitive to the pituitary hormone, as Krogh apparently found them to be in the frog. It may be, on the other hand, that their tone is normally under such efficient control of other kinds that they are not further affected. In favour of the latter view is the restorative action of pituitary extract on the circulation in certain conditions of threatened circulatory failure. In a private communication, Prof. Krogh has informed me of some recent observations by one of his colleagues, who produced the characteristic shock-like condition with histamine, and found that he could restore the circulation by administering pituitary extract.

To summarise this evidence, we may say that there must be chemical agents for the maintenance of capillary tone, which can supplement, or even replace, the nervous mechanism. Of such agents we have evidence of two, produced by the suprarenal medulla and the posterior lobe of the pituitary gland respectively. Concerning the former, adrenaline, we have good evidence that it is constantly being secreted into the blood, and that this secretion can be accelerated in accordance with the need for its action. Concerning the pituitary pressor principle we have no such clear evidence, and we shall have to discuss later the grounds for crediting it with a hormonal function at all, at any rate in

normal mammalian physiology. In these circumstances it is hardly worth while to discuss whether it preferentially affects the capillaries of certain organs in the mammal, as, according to Krogh's experiments, it appears to do in the frog. My own reading of the evidence leaves me rather with the impression, that adrenaline has the more general importance in this direction in the mammal, and that the pituitary principle, if active at all, acts rather by reinforcement of the adrenaline effect.

In concluding what I had to say about the control of tone in the capillaries, I have already entered upon the proper subject of this lecture, in which I have undertaken to discuss the active principles of the pituitary body. You will be aware that this gland—an organ credited with an extraordinary multiplicity of functions—consists, like the suprarenal gland, of portions which are embryologically, morphologically, and physiologically distinct.

Embryologically it consists of two main portions, formed respectively from a pouch pinched off from the roof of the stomodæum, and by an outgrowth of nervous tissue from the floor of the third ventricle. This nervous outgrowth develops into a funnel-shaped stalk or infundibulum, ending in a club-shaped mass, the so-called *pars nervosa* of the adult pituitary body. The stomodæal portion becomes invaginated to invest the nervous portion, and develops into the *pars anterior*, the main glandular portion of the pituitary, and the *pars intermedia*, which is a thinnish layer of cells investing the *pars nervosa*,

and reflected on to the *pars anterior*. The *pars nervosa* and its investment of *pars intermedia* are together commonly spoken of as the *posterior lobe*—the material from which is made the now familiar pituitary extract used in therapeutics; while the *pars anterior* with its thin lining of *pars intermedia* forms the *anterior lobe*, separated from the posterior lobe by a cleft, which is the remnant of the primitive cavity pinched off from the stomodæal pouch.

It is the anterior lobe which, under the microscope, has the glandular appearance; the posterior lobe, except for the glandular investment of *pars intermedia*, appears to consist of degenerate nervous tissue, and has little cellular structure. Looking at the histology of these different parts, one would be inclined to predict that, if hormones were produced by such an organ, it would be from the glandular-looking anterior lobe that we should be able to extract them. As a matter of fact, it is doubtful whether anybody has succeeded in extracting hormones from the anterior lobe,[1] while the posterior lobe, and especially the remarkably featureless nervous part of it, yields an extract which shows an immediate physiological activity, of extraordinary intensity, on several distinct bodily functions.

Though the active principle or principles have never been extracted from the anterior lobe, in such

[1] The lipoid substance prepared from the anterior lobe, and named "*tethelin*" by Brailsford Robertson, is stated to have specific action on growth; but its claims to chemical individuality and to specific activity are still the subject of controversy.

a form that their action can be demonstrated beyond question, there can be little doubt that it does exercise a function of internal secretion. The best evidence is obtained from disease involving its overgrowth or excessive action, causing the characteristic enlargement of the bones and fleshy parts of the face and extremities known as *acromegaly*, or the general overgrowth which produces gigantism.

The effects of extirpation, as described by different authors, are rather confusing. Some maintain that the anterior pituitary lobe is essential to life, others that it can be removed without causing definite symptoms. It is difficult to be quite sure, moreover, which among the symptoms of deprivation described are due to the loss of the anterior lobe, which to the unavoidable injury to the posterior lobe. I cannot attempt to put the whole of the rather conflicting evidence before you, and must be content to record my own impression that its balance is in favour of the conclusion that removal of the anterior lobe by itself in *young* animals greatly retards growth, stops sexual development, and produces a kind of infantilism, in some points similar to that seen in man as a result of pituitary deficiency. There are other symptoms which have been described as following removal of the anterior lobe, and also seen in naturally occurring deficiencies, which are more probably due to accidental injury to, or concurrent deficiency of the posterior lobe, in connection with which I shall speak of them.

When we come to the posterior lobe, we enter a

field of easier experimental enquiry, in that we are dealing with an organ which yields an immediately active extract. The action was discovered by Oliver and Schafer, who, after they had observed the extraordinary activity of extracts from the suprarenal gland, made a general survey of the glands of the body, injecting decoctions of them and looking for actions on the blood-pressure. The only extract showing anything comparable to the sensational activity of that from the suprarenal gland, was that from the pituitary body, which also had a very powerful pressor action. Howell first observed that this active pressor principle was limited to the posterior lobe, and that, when one effective injection had been given, the animal remained for a long time relatively insensitive to further similar injections. Schafer and Vincent confirmed Howell on these points, and first noticed that, with a second or subsequent injection, the characteristic pressor effect was not merely in abeyance, but was replaced by a depressor effect.

It will be convenient to deal with this depressor action at once. Schafer and Vincent observed that a depressor constituent could be extracted from the gland substance with alcohol, and the latest evidence, obtained by Hogben and Schlapp in Schafer's laboratory, seems to me to prove quite clearly, that if such alcoholic extraction is carried out completely, it will remove the whole of the depressor substance and leave a purely pressor principle behind, an extract from a gland so treated now producing a large

pressor effect with the first injection, and a small, but still purely pressor effect with a subsequent injection, given as soon as the effect of the first has subsided, without any depressor phase at any stage. The depressor substance extracted by alcohol will produce an apparently pure depressor effect in a cat under ether, while in a spinal cat it will often produce a fall followed by a rise of arterial pressure. Under similar conditions histamine in small doses will produce practically identical effects, and there is evidence that the secondary rise, following the essential depressor action, is due to output of adrenaline from the suprarenal glands. Indeed, I think there can be practically no doubt that this depressor constituent is not a specific pituitary principle at all, but simply one of the substances with histamine-like action, which, as I mentioned before, can be obtained from almost all organs of the body. For brief reference we will call it the histamine-like constituent, and return to the truly specific actions of the pituitary extract.

One of these, the powerful pressor action, we have already dealt with. It is due essentially to vaso-constriction, the rate and efficiency of the heart-beat being little, if at all, affected directly. The action appears to have no relation to innervation; after sufficient ergotoxine has been given to paralyse completely the pressor action of adrenaline, and replace it by a depressor action, the pituitary principle still produces its pressor effect unimpaired. One would suppose from this that its action was a direct one on

the contractile cells of the vascular walls—the plain muscle fibres of the arteries and the Rouget cells, as we must now add, of the capillaries. If attention is restricted to the mammalia and the amphibia, one finds that the action on the plain muscle of almost any organ is to stimulate it to contraction. In birds, on the other hand, as Paton first showed, the pituitary extract anomalously causes a *fall* of arterial blood-pressure, apparently due to vasodilatation. In the present state of our knowledge it is impossible to say whether the principle causing this depressor effect in the bird is identical with that which produces the pressor effect in mammals. Of one thing only can we be definitely certain concerning this effect, viz. that it is not due to the alcohol-soluble, non-specific, histamine-like substance. When this is completely removed, and an extract obtained with a purely pressor action on mammals, it still acts as a pure depressant for the circulation of the bird.

A second action of the pituitary extract, also discovered by Schafer and his co-workers, is an effect on urinary secretion. In an anæsthetised cat, dog, or rabbit, in which the secretion of urine is normally a slow process, intravenous injection of a dose of the extract will produce an immediate slowing or complete arrest of the flow, lasting till the rise of blood-pressure has passed its maximum, and then giving way to a pronounced acceleration, accompanied by a large increase in the volume of the kidney.

I suggested, some years ago, that this particular effect might also be due to the principle acting on

the blood-vessels, and secondary to the vascular change, the primary inhibition corresponding with a constriction of the arteries of the kidney, together with those of the rest of the body, the more prolonged acceleration, accompanied by expansion of the kidney, being explained by an earlier decline of the vascular effect in the kidney than in other organs, so that a larger proportion of the circulation passed through it. Of recent years this idea has been substantiated by evidence from several different investigators, showing that the diuretic action is proportional to the increased blood flow through the kidney, and is not accompanied by an accelerated metabolism of the organ. We shall see that there is other evidence for regarding the diuretic effect as merely incidental to the pressor action. Meanwhile I will warn you against confusing it with a quite different, and probably much more important action of the pituitary extract on the kidney itself, to which we must give separate attention presently.

Another action of the pituitary extract, which forms the basis of its commonest use in therapeutics, was observed by myself and independently by v. Fränkl-Hochwart and Fröhlich in Vienna, namely, its intense stimulant action on the plain muscle of the uterus. It is necessary, in connection with this action, to make it clear that we are dealing with a true, specific, active principle of the pituitary gland, and not with the non-specific, histamine-like constituent. There has been some confusion on this point, and it is possible that it still complicates some of the evidence.

Prof. Abel, of Baltimore, who at one time claimed to have identified this so-called oxytocic pituitary principle with histamine, now agrees with other observers in finding that the true pituitary principle is an enormously more active, as well as a much more complex and less stable substance. This action on the uterus lends itself particularly well to quantitative estimation, and by its use we have obtained some idea of the astounding activity of the pituitary principles. When we found, some fifteen years ago, that histamine would cause powerful contractions of the isolated uterus when diluted to 1 part in 100 millions, we seemed to have reached almost a limit of credible physiological activity ; but we are far beyond that now. Prof. Abel has already made a small quantity of a preparation from the pituitary posterior lobe which is more than 1,000 times as active as this, acting therefore in a dilution of 1 part in 100 thousand millions—or 1 in 10^{11}. I find no good reason for believing that, even so, he has obtained, as he supposes, a nearly pure principle ; indeed, I think there is very good reason for believing otherwise ; but the activity is, in any case, sufficiently impressive. The question as to whether the stimulation of the uterus and that of the blood-vessels are due to the same or to different principles we may leave conveniently, till we have mentioned yet other actions.

Another action discovered by Schafer and his co-workers is the galactogogue action—a rapid outpouring of milk from the lactating mammary gland —when the pituitary extract is administered intra-

venously. There is no evidence of accelerated *formation* of milk ; the quantity obtainable from a milch-cow or goat over a period cannot apparently be increased by injecting pituitary extract. It is rather a case of expression into the ducts of milk already formed, so that it is more immediately available ; and if the flow of milk from an incision made into the gland is recorded, as in Schafer and Mackenzie's original observation, the rate is immediately accelerated. It is doubtful whether the action has any serious physiological importance, and doubts have even been raised recently as to whether it is a specific pituitary effect, or due to the non-specific, histamine-like constituent of the ordinary extract.

There is another curious action of the extract obtained from the posterior lobe, which I have already mentioned incidentally, in connection with Krogh's observations on the capillaries, and which has been described most completely by Hogben and Winton. This is its action on the melanophores of the frog, which are caused to expand, so that the skin becomes dark in colour. If the pituitary body, or only its posterior lobe, is removed from a frog, the animal becomes very pale in colour, and remains so even when kept wet in a dark place. Removal of the anterior lobe alone produces no effect of this kind. Expansion is caused by injecting an extract of posterior lobe. The reaction of the melanophores to this principle is extremely delicate ; Fenn, in my laboratory, found that an extract of 1 part of the whole dried posterior lobe in 10^{10} parts of Ringer's

solution was enough to produce a distinct effect with perfusion. The figure is the more impressive in that Hogben and Winton found that this particular activity of the extract was contributed mainly, if not entirely, by that small part of the posterior lobe which is formed by the *pars intermedia.*

Then there is the important *antidiuretic* action which the pituitary extract exerts in the normal, unanæsthetised animal or man, checking the diuresis following ingestion of water, and in cases of *diabetes insipidus* checking the abnormal flow of dilute urine, and rendering the kidney function for the time approximately normal. I have purposely separated this action widely, in my survey, from the brief diuretic action seen in the anæsthetised animal, in order to emphasise the fact that there is as yet no good reason to suppose that they are connected. We have seen that the diuretic action is conceivably a by-product of the vasomotor effect. There is no similar reason to suppose that the antidiuretic action has any relation to it. Very significant as to its meaning is the recent work of Starling with Verney and with Eicholtz, who found that the isolated kidney, perfused with blood from a heart-lung preparation, secreted a urine which became progressively dilute, and especially poor in chlorides as the experiment proceeded. Addition of a small quantity of pituitary extract to the circulating blood promptly reduced the volume of urine secreted, and increased its concentration of chlorides in higher ratio. It seems clear that a pituitary principle intervenes

somehow in the change of water and chloride contents in the kidney tubules, which converts a glomerular filtrate into true urine.

The last action which we have to notice is a controlling effect which the extract appears to exercise on carbohydrate metabolism, of such a kind that it acts antagonistically to the production of hyperglycæmia by adrenaline, on the one hand, and to the production of hypoglycæmia by insulin on the other. I shall have to deal with this action in a later lecture, when speaking of insulin, and merely to mention it will suffice for our present purpose.

Altogether, then, no less than eight different kinds of physiological effect have been discovered, all of which the extract of the pituitary posterior lobe produces, when it is injected into a vein, or otherwise suitably brought into contact with the reactive structures. The question naturally arises, whether these represent different aspects of the activity of a single, multivalent principle, or whether several principles are present, each responsible only for one, or for a few, of these effects. The question is one upon which opinion is still sharply divided. To judge it fairly we must consider arguments based on the distribution of the different kinds of activity in the gland substance, and those which have been founded on the chemical behaviour of the active material.

It is generally agreed that, in perfectly fresh specimens of the pituitary body, the different forms of activity, which we are considering, are con-

fined to the posterior lobe. You will remember, however, that the posterior lobe, though consisting mainly of *pars nervosa*, contains also, as an investing layer, the *pars intermedia*. Of the eight different recorded activities of extracts from the posterior lobe, we can dismiss the depressor action as probably not specific. Nothing appears to be known definitely as to the distribution of the antidiuretic action, or of the controlling action on carbohydrate metabolism, as between the two embryologically different parts of the lobe. Concerning all the others evidence on this point is available. It may be stated that only one of these other five activities is found predominantly in the *pars intermedia*, namely, the expanding effect on the frog's melanophores, extracts of pure *pars nervosa* being relatively very weak in this action. Concerning the other four, all evidence is concordant in finding that they are much more strongly represented in the *pars nervosa* than in the *pars intermedia*. There are differences of detail. Herring, who first recognised the distinction between these parts of the posterior lobe, and explored the distribution of the different activities between them, found no pressor or diuretic action in extracts from *pars intermedia*, and relatively weak oxytocic and galactogogue actions. Others have found that the *pars intermedia* yields traces also of pressor and diuretic activity. But the general distinction is clear. Only the melanophore-expanding activity is chiefly to be found in the *pars intermedia*, the other four being preferentially localised in the *pars nervosa*.

As to the interpretation of these findings opinions differ. There is nothing in the physiological evidence to exclude the possibility that the pressor, diuretic, oxytocic, and galactogogue activities may be confined to the *pars nervosa*, and the melanophore-expanding principle to the *pars intermedia*, during life; the traces of these activities found in the alternative lobes might quite reasonably be attributed to *post-mortem* diffusion. On histological grounds, however, most writers on the subject have been inclined to regard the *pars intermedia* as the place of origin of all, and to suppose that the principles obtained by extraction from the *pars nervosa* are simply accumulated there, or perhaps receive there the finishing touches to their activity. If we confine our attention to the physiological facts, the known distribution of the different activities may be tabulated as follows:

	Pars intermedia.	*Pars nervosa.*
Pressor . . .	− (+)	+ + +
Diuretic. . . .	− (+)	+ + +
Oxytocic . . .	+	+ + +
Galactogogue . . .	+	+ + +
Melanophore . . .	+ + +	+

Leaving aside the question as to the origin of this unequal distribution, it is fair to conclude from it that at least two, and probably at least three active principles are concerned in producing the different effects.

Now as to their chemical nature. The study of this has provided the main battle-ground, between those who believe that one principle is responsible

for all the different actions, and those who believe that at least three must be concerned. The "unitarians," such as Prof. Abel, base their view on the similarity in chemical behaviour of the substances responsible for the different actions, and the difficulty of separating them by chemical means. Let it be granted that they appear to be due to *similar* substances, all apparently of a relatively simple polypeptide nature. All are resistant to peptic, but easily destroyed by tryptic digestion, or by dilute caustic alkali at room temperature. All are diffusible through collodion. Prof. Abel has shown that they seem to cling together through a series of precipitations. Unfortunately for the unitarian position, his evidence proves too much ; for his final, most active preparation, which he regards as a nearly pure preparation of the single active principle, retains the depressor and melanophore-expanding actions, which we strongly suspect, on other grounds, to be due to different substances.

Prof. Clark has shown that a collodion membrane of a certain density will hold the melanophore principle back and let the oxytocic through. My colleague Dr. Dudley showed some years ago that a neutral solvent, like wet butyl alcohol, preferentially extracts the oxytocic principle from watery solutions, leaving the pressor principle mostly behind. This was confirmed by Prof. Fenn, who found, as we should expect on other grounds, that the diuretic and pressor activities stayed together, being less easily extracted by butyl alcohol from the watery

solution, in which also the principle dilating the frog's melanophores was mostly left behind. The one principle, of those for which tests were made, which was differentiated from the others by the relative ease with which butyl alcohol would extract it, was the oxytocic or utero-stimulant principle. Unfortunately the behaviour of the galactogogue in this respect has not yet been investigated. If we put together the evidence of different kinds, it appears necessary to assume that there are at least three different principles.

1. { Pressor. / Diuretic.
2. { Oxytocic. / Galactogogue.
3. Melanophore-expanding.

? Antidiuretic.
? Sugar mobilising.

Prof. Abel's experiments, showing that the different forms of activity stick closely together through a series of precipitations, are regarded by him as establishing the identity of the different principles. In any case such failure to separate the active principles could not establish more than a *prima-facie* presumption of identity. Even that presumption loses much of its support, when one realises that his precipitations were in almost all cases due to the adsorption of the active principles on to amorphous precipitates of non-specific materials. And, in any case, the record of any number of failures to separate loses all its evidential value, in face of one demonstration that the

different forms of activity can be even incompletely separated, either by their distribution in the gland, or by selective filtration through a membrane, or by the selective action of a solvent.

The last question we have to deal with, is as to whether these substances act as true hormones, and, if so, as to the channels by which they reach the circulation. I think we ought to attach *some* weight to the argument, that it is hardly conceivable that an organ like the pituitary body, which is known to have functions of internal secretion from one of its lobes, should store up, in its other lobe, substances having such specific and intense activities, related to important bodily functions, without their having any significance for the normal control of those functions. On the other hand, with regard to the vascular stimulant, practically the only evidence we have of its normal entry into the circulation, or of its share in the control of vascular tone, is Krogh's evidence of the loss of capillary tone and resulting œdema in the frog, following removal of the pituitary posterior lobe. Evidence of the same kind applies to the melanophore stimulant; and we are left wondering what can be the function, in the mammal, of a principle concerning which we only know that it causes expansion of melanophores in the frog. Similarly perplexing is the evidence (Herring) that, of these various activities of the pituitary body, only the stimulant action on the uterus and the galactogogue effect are discoverable in extracts from the pituitary body of the elasmobranch fish.

There is, nevertheless, some direct evidence of the hormonal activity of the oxytocic constituent of the mammalian pituitary. It is to some extent involved with the view, first put forward by Cushing on histological grounds, supported by certain physiological evidence, that these pituitary principles reach the circulation, not by direct absorption into the blood, but by excretion, through the lumen of the infundibular stalk, into the cerebrospinal fluid in the third ventricle of the brain. Profs. Dixon and Marshall, of Cambridge, with more convincing physiological records, have carried the story a stage further, so far as the internal secretion of the oxytocic principle is concerned. They find that the cerebrospinal fluid, drained from the subcerebellar cisterna of an anæsthetised dog, does not normally contain a uterine stimulant in significant quantity. When, however, extract from an ovary of an animal, killed during œstrus or at the end of pregnancy, is injected into the dog's circulation, a substance acting like the pituitary oxytocic principle appears in the cerebrospinal fluid. They regard this as indicating a mechanism, concerned in bringing about the contractile activity of the uterus at the œstral period or at the termination of pregnancy. That it cannot be the only influence concerned in determining the onset of labour, is made clear by the fact that direct injection of pituitary extract itself, even in large doses, will not bring about the termination of a pregnancy before its due time. There seem to be some links in the chain still missing. But, so far as they go, these

observations do seem to support the views that the oxytocic principle is a true hormone, and that, at least in part, it reaches its destination by the indirect route of the cerebrospinal fluid.

The other evidence, in favour of the hormone action of some of these pituitary principles, comes from the effects of disease or of operative removal. As I said earlier, the distinction between anterior and posterior lobe is not very clear in this connection. But when we find, on the one hand, that the condition of *diabetes insipidus* is associated with degenerative states of the pituitary, produced in a few interesting cases by bullets lodged in the *sella turcica* and pressing on the gland ; and, on the other hand, that the condition is promptly, though temporarily, relieved by injecting extract of the pituitary posterior lobe, just as the same extract restored to the kidney the function of secreting a normally concentrated urine in Starling's experiments ; then the case seems almost as clear as any other in endocrinology, in favour of a hormonal control over part of the complex kidney function. The same is true with regard to the regulating function of the pituitary posterior lobe on carbohydrate metabolism—based apparently on an antagonism to excessive function of the pancreatic insulin, which we shall consider more fully in a later lecture.

But during recent years the significance of all this evidence has been called into doubt, by evidence from several laboratories (including some from the laboratory of Cushing, who was earlier a leading

exponent of the significance of these pituitary deficiencies), to the effect that all these symptoms attributed to pituitary deprivation—polyuria, abnormal sugar tolerance, obesity, and infantilism—can be produced by puncturing the floor of the brain in the region of the *tuber cinereum*, without injuring the pituitary body at all. It is suggested by these observers, that the supposed effects of injury or removal of the pituitary, by disease or operation, are fallacious, have nothing to do with hormone action, and are due to concurrent and almost unavoidable injury to nerve centres in the neighbouring hypothalamus. I feel bound to say that to me the independent evidence of hormone action appears to be too strong to be lightly dismissed ; and, though the position is obscure, and badly needs clarifying by further evidence, it seems just as reasonable, in the meanwhile, to suppose that puncturing the *tuber cinereum* in some way, as yet unknown, interferes with the internal secretory function of the pituitary posterior lobe, as to suppose that all the observed results of disease or injury to this part of the gland are really due to concurrent injury of the brain, and to regard the restorative effects of injecting pituitary extracts as merely accidental. Every research-worker knows that Nature may sometimes appear to be an artist in the construction of " booby-traps " ; but the location so near to the significant nerve-centres, that these are unavoidably injured in its removal, of an organ containing an intensely active principle which, on injection, can

remove with precision the effects of the nervous injury, cannot yet be dismissed as a merely misleading coincidence.

LITERATURE

Literature concerning the active principles is given in Prof. Abel's Harvey Lecture, *Bull. Johns Hopkins Hosp.*, xxxv, p. 305, 1924. Two monographs in Spanish by B. A. Houssay (*La Accion fisiologica de los Extractos Hipofisarios*, Buenos Aires, 1918 and 1922) give exhaustive bibliographies. For the general morphology and physiology of the gland, see Swale Vincent, *Internal Secretion and the Ductless Glands*, 3rd edition, 1924.

LECTURE III

THE PANCREAS AND INSULIN

KNOWLEDGE of the internal secretion, by which the pancreas controls carbohydrate metabolism, has been the subject during recent years of a development so rapid and romantic, and so fruitful in practical therapeutic result, that it has struck the imagination of the public in all civilised countries with a force probably without parallel in the history of physiological discovery. I think it will be interesting and instructive to trace in outline the history of knowlege and investigation in this field from an earlier date, in order that we may remind ourselves of the position which had been reached prior to 1921, and realise the extent and significance of the change.

Up to the year 1889 nothing was known of a connection of the pancreas with carbohydrate metabolism. Early attempts, indeed, had been made, from the seventeenth century onwards, to extirpate the pancreas, but these had either proved rapidly fatal, from unrecognised causes, or were incompletely performed. Meanwhile the work of Claude Bernard had concentrated attention on the nervous control of the output of sugar from the liver, showing that indirect stimulation of the sympathetic nerve supply through the *medulla oblongata*, in the well-known

" piqure " experiment, caused transformation of the liver glycogen into sugar, with resulting increase of the proportion of sugar in the blood, and its excretion in the urine. The impression was naturally produced that *diabetes mellitus* was probably due to a disturbance of this nervous control; and, if occasionally the condition was found to be associated with a sclerotic condition of the pancreas, this was easily accounted for by involvement of the solar plexus. So prepotent were these ideas, connecting diabetes with anomalies of the nervous control, that the veteran Pflüger, till his death, refused, in the face of the most direct evidence, to admit that the diabetes following operative removal of the pancreas had any connection with the loss of a pancreatic function, and persisted in attributing it to accidental injury of the nerves to the duodenum.

To everyone but Pflüger, however, the position was changed completely by an almost accidental observation made by v. Mering and Minkowski in 1889. v. Mering was apparently studying fat-absorption, and, with the object of investigating the effect of the pancreas on this function, he asked his assistant, Prof. Minkowski, to endeavour to remove the pancreas completely from a dog. The operation was successful, and it was noticed that the dog, on the following day, was passing greatly abnormal quantities of urine, and that it displayed an insatiable hunger and thirst. Investigation of the urine showed that it contained a high proportion of dextrose. Apparently De Dominicis had made a similar obser-

vation independently, and had embodied his results in a thesis. Further studies by Minkowski showed that the condition produced by this operation was, in practically all respects, identical with a *diabetes mellitus* of extreme severity. The blood-sugar on a full diet rose from the normal proportion of about 0·1 per cent to as much as 0·7 or 0·8 per cent, with 8 to 10 per cent of glucose in the urine. The only cardinal symptom of severe human diabetes which was frequently missing, and never strongly in evidence, was the ketonuria —the presence in the blood and the urine of acetone itself, and of abnormal fatty acids, β-hydroxybutyric and acetoacetic acid, which readily yield acetone. The absence or irregular appearance of this symptom, however, is probably due to the acuteness and rapidly fatal issue of the diabetes produced by pancreatectomy, and does not materially weaken the evidence in favour of regarding the experimental condition as a true diabetes.

That a hitherto unsuspected function of the pancreas was involved was perfectly clear. Both Bernard and Schiff had shown much earlier that the pancreatic duct could be blocked, and its known function of secreting a digestive juice thereby abolished, without any serious impairment of the animal's health. Of those who repeated and confirmed Minkowski's observation of the diabetes following extirpation, Lépine was apparently the first to suggest that it indicated an internal secretory function of the pancreas, controlling in some way the normal metabolism of carbohydrate. The correctness of this

suggestion was soon established by Minkowski and by Hédon, who showed that a graft of a portion of the pancreas could be made under the skin, and that, when this had formed vascular connections, the remainder of the organ could be removed without producing any sign of diabetes, which appeared as usual, however, if the grafted portion was subsequently removed. Such a result could only be explained in one of two ways: the pancreas must either produce an internal secretion needed by the body for dealing with carbohydrate, or must constantly remove from the blood a substance which impeded the normal metabolism. A similar alternative, it may be remarked, has arisen in practically every instance in which the function of a glandular organ has been revealed by its ablation; and I cannot recall any such case, in which the later evidence has not decided the issue in favour of an internal secretion, and against a detoxicating action.

Precision was given to the theory of an internal secretion by the suggestion, made by Schafer and by Diamare, that it might be produced by the peculiar islets of cellular tissue, irregularly scattered among the secreting acini of the pancreas, and first described in 1869 by Langerhans. There was not much to support the suggestion at the time, except that these structures had no apparent relation to the external secretion of the digestive ferments, and that they were well supplied with large blood capillaries, which seemed appropriate to an endocrine function. The islets, like the externally secreting acini, originate as

buds from the primitive pancreatic duct; indeed, according to Laguesse, the pancreas in its earliest development consists entirely of solid outgrowths from the duct wall, which he calls "primitive islets," from which the secretory acini and the islets become later differentiated. Several observers, myself among them, have seen appearances, from time to time, which have suggested the possibility of modification in the reverse direction, that is, of the formation of new islets in the adult pancreas in the place of the secreting acini. The balance of the evidence now available seems to me, on the whole, unfavourable to such an occurrence; on the other hand, I think it is more generally admitted that new islets may be formed by proliferation of duct epithelium. Fortunately division of opinion, on this question of the independence and relative permanence of the two kinds of pancreatic tissue in the adult, does not imply a similar division of opinion as to the function of the islets. Schultze long ago provided clear evidence as to their endocrine function, by showing that, when the pancreatic duct had been blocked with paraffin, and time given for degeneration, the sclerotic remnant of the pancreas, which still sufficed for the maintenance of a normal carbohydrate metabolism, consisted of dense connective tissue, with only islets embedded in it. And whether we suppose that these islets are all survivors from those which existed before the operation, or have been partly formed, during the process of degeneration, from the acini, or by new budding from the ducts—and the evidence on

the point is not quite clear—the conclusion of chief physiological importance was clearly established, namely, that a pancreas reduced to islet tissue alone can perfectly well perform the endocrine function of the gland.

Further light was thrown on the endocrine function of the islets by the histological work of Lane and of Bensley, and by the experimental work of Allen, in which their methods were utilised. Histologically it was found possible, by the use of special staining methods, to recognise two types of cells in the islets, named the α- and β-cells, and differentiated by the staining properties of their granules. Allen worked with dogs in which only a sufficient remnant of pancreas had been left to keep the metabolism normal on a carefully restricted diet. By administering carbohydrate in excess of their tolerance, the resistance of such animals could be so broken down that not merely a temporary glycosuria, but even a permanent diabetes might result. Allen was able to show that such a breakdown of tolerance was accompanied by changes in the β-cells only. These lost their characteristic granulation and became swollen. They might recover and resume their normal aspect, or the swelling might terminate in disintegration and disappearance. By their fate, apparently, the issue was determined, as between temporary breakdown of tolerance and permanent, fatal diabetes.

But while histological evidence for a pancreatic hormone thus progressed to the presumptive identification of the cells producing it, and even of the

granules in which they held it ready for secretion, attempts at its chemical separation from the pancreas, and its preservation for use in experiment or in therapeutics, continued to fall short of success. There were hints of success from time to time, as in Starling's work with Knowlton and with Evans, on the increased use of sugar by the heart from a diabetic dog, and the rise of the respiratory quotient of such a heart when an acid extract of pancreas was added to the circulating blood ; or Maclean and Smedley's finding that the heart from a diabetic animal would use no glucose at all when perfused with Ringer's solution, but began to do so when pancreatic extract was added. Perhaps the clearest evidence of all, in favour of a pancreatic hormone, was provided by Clarke, of Baltimore, who showed that an isolated dog's heart would remove glucose from Ringer's solution much more rapidly, if the solution was first allowed to perfuse the vessels of the pancreas. But the net result of all such experiments was to create the impression that the pancreatic hormone was either secreted as quickly as formed, so that no store was available for extraction, or, if stored, was so unstable outside the organ, that the possibility of its separate preparation was remote. Looking back from the present position, it is difficult for anyone who has not had direct experience of the problem to understand the reason of the failure, or, at best, of the success so partial and capricious as to be almost more baffling than failure, which attended the efforts of so many experienced observers. E. L. Scott

actually extracted pancreas with the solvent, diluted and acidulated alcohol, which is most commonly employed to-day for the extraction of insulin in manufacture ; yet he was obliged to report that his attempt was unsuccessful, and his solution apparently devoid of the desired activity. The explanation may become a little clearer as we proceed.

Banting and Best, in embarking on their now famous investigation, started from premises, of which the correctness is not proved beyond doubt by their success. They supposed that the pancreatic hormone might have been lost by previous workers, who had essayed its extraction, owing to the destructive action of trypsin extracted with it.

In one respect their assumption was correct ; we know now that insulin is very readily destroyed by trypsin. In another respect it presents difficulty ; the perfectly fresh and cleanly removed pancreas, such as many workers had used, contains no trypsin. Right or wrong in itself, the idea led them to try an experiment of great importance. They blocked the pancreatic duct of a dog, allowed time for complete degeneration of the acini, and then made an extract, with cold Ringer's solution, from the sclerotic, islet-containing remnant, and tested the effect of injecting this extract into a dog rendered diabetic by extirpating its pancreas. The result was a success which nobody could have anticipated, whom experience had habituated to the long history of failures. The injection caused a prompt fall of the blood-sugar and reduced the glycosuria. By repeated injections

the animal could be kept in a relatively normal condition, and when glucose was administered with the extract, instead of being entirely excreted, as Minkowski had observed in his dogs, it was partly retained and metabolised.

It was obviously impossible to depend upon extracts from pancreas degenerated as the result of operative procedure, if the pancreatic hormone was to be used in practical therapeutics, or its action subjected to a thorough experimental investigation. But the possibility of obtaining it and keeping it in artificial solution had, once for all, been demonstrated ; the hormone was proved to be present in sufficient quantity, and its preparation from normal pancreas became an obvious possibility, if only the right method could be found. After some preliminary experiments with fœtal pancreas, taken at a stage of development at which it should still be free from trypsinogen, it was found possible to obtain an active extract even from ordinary adult pancreas from the slaughter-house,using dilute alcohol (80 per cent) as the extracting solvent. After that it became merely a question of improving the yield and purifying the crude extract from inert or irritating constituents. One of the first improvements, published from Shaffer's laboratory, was the strong acidulation, with mineral acid, of the dilute alcohol used for extraction. This amounted to the employment of practically the same solvent as Scott had tried in vain some years before.

Some of the properties of insulin were revealed

by the processes elaborated for its purification. It is soluble in water at neutral reaction and in concentrations of alcohol up to about 80 per cent. Above that concentration it begins to be thrown out of solution and is fairly completely precipitated by alcohol of 95 per cent strength. It is thrown out of solution by saturation with ammonium sulphate. From neutral or slightly acid watery solution it can be completely precipitated by picric acid. The picrate so obtained is insoluble in water or in perfectly dry alcohol or acetone, but dissolves fairly readily in alcohol or acetone containing a little water, and from such a solution the insulin can again be precipitated as a hydrochloride. Perhaps the most remarkable and characteristic of its properties is its relative insolubility in water if the reaction is adjusted to just over pH 5. If the reaction is shifted slightly to either side of this point, to pH 4 or pH 6·5, the precipitate goes again into solution. The further the purification is carried, by any of the processes of fractionation available, the more complete becomes this precipitation at the isoelectric point, and the narrower the range within which it occurs. In acid solution insulin is relatively stable to heat, and under such conditions will survive heating to 100° C. for at least half an hour. In alkaline solution it is much less stable, and is rapidly destroyed even at body temperature.

With respect to this influence of reaction on its stability, and to many others of the characteristics mentioned, insulin resembles other hormones, such

as secretin and the pituitary active principles. That it is a more complicated substance than these latter, however, is suggested by the fact that it is rapidly destroyed, not only by tryptic but also by peptic digestion.

The behaviour seems to indicate a peptide structure. Some of the colour reactions, characteristic of certain amino-acids, while given by the cruder product, disappear as the specific activity is concentrated into a smaller and smaller weight of material. The Millon reaction for tyrosine, and the glyoxylic acid reaction for tryptophane can thus be eliminated. Macleod and his colleagues report that they obtained an active preparation from skate's pancreas in which even the biuret reaction was no longer present. Further evidence on that point would seem desirable. On the other hand, there are reactions, suggestive of other amino-acids, which seem to grow stronger as purification proceeds. Such are Pauly's reaction with diazobenzenesulphonic acid for histidine, and the reaction for organically combined sulphur, which might be taken to indicate cystine as a constituent. It may be noted that the histidine diazo-reaction seems to maintain a similarly close association with the physiological activity in the case of the pituitary active principles. But, in both cases, these chemical indications are of uncertain significance, in view of the fact that we are not dealing with pure substances, and have no knowledge of the proportion in which the true active principles are present in the material examined. Insulin, like the pituitary principles, is

very strongly adsorbed by substances precipitated out of the solution containing it. The problems presented by attempts to purify these hormones have analogies with those encountered in attempting to purify enzymes. Properly speaking, we have no right to attribute certain solubilities to insulin, or to say that insulin itself is precipitated at an isoelectric point in the neighbourhood of pH 5. All that the facts strictly justify us in concluding is that a particular peptide substance has such and such solubilities, and is precipitated at its isoelectric point, or by picric acid, and that insulin accompanies it into solution and is carried with it into precipitation. It may be suspected that it was this tendency to adsorption on to precipitates out of watery solution, even more than its sensitiveness to trypsin, which so long frustrated efforts to extract this hormone. The acid added to the extracting alcohol, with the intention of inhibiting tryptic action, has probably more importance in bringing the reaction well to the acid side of pH 5, and allowing the insulin to pass into solution. Dudley found, indeed, that adding sodium bicarbonate, ignoring the danger of tryptic destruction, and raising the pH to the neighbourhood of 7, was at least as effective as acidulation in increasing the amount of insulin extracted.

As to the origin of insulin from the Langerhans islets, recent evidence has been rather confusing. Macleod and his co-workers, indeed, seemed to have established the connection more definitely by experi-

ments on fish. In certain teleosteans compact clumps of tissue occur, in relation to the diffuse pancreas, which consist almost entirely of a structure resembling the islets of other species, with at most a thin covering of pancreatic acini. These so-called "principal islets" were found to give a high yield of insulin in proportion to their weight, while the neighbouring acinous tissue appeared to yield none at all. Swale Vincent and Dodds have confirmed the high yield from the principal islets, though they still obtained some insulin from what appeared to be pure acinous tissue. Still more difficult, at first sight, to reconcile with the theory of the islets as the sole place of origin, is Dodds's statement that insulin can be obtained, by suitable methods, in good yield from other organs, such as the kidney. Winter and Smith obtained from yeast a preparation having apparently an insulin-like action, while Collip has obtained substances producing, on injection, a curiously delayed lowering effect on the blood-sugar, from a variety of vegetable materials, such as the shoots of green plants. It may be recalled, in this connection, that it was shown some years ago that injection of an extract from spinach would produce a secretion of pancreatic juice, not unlike that produced by an injection of true secretin. I do not think that it would be reasonable to conclude from this observation that the hormone secretin is widely distributed in the vegetable kingdom. Concerning Collip's so-called "glykokinins" we do not even know that their action is a direct one. The effects were demon-

strated on normal rabbits, and the delay in their appearance suggests that they might be secondary to an effect on the pancreas, exciting this organ to the output of true insulin. I do not think, however, that the same interpretation could be applied to the puzzling facts concerning the possibility of preparing insulin from other mammalian organs than the pancreas, including the blood. Banting and Best found that the blood from a normal rabbit, subjected to the process used for obtaining insulin from the pancreas, would yield enough insulin to cause complete disappearance of blood-sugar from several other normal rabbits, into which it was injected. Injection of the equivalent amount of untreated blood had no effect of the kind. So far as the evidence goes, therefore, it suggests that, apart from the store in its place of origin in the pancreatic islets, insulin is normally circulating in the blood, and deposited in other organs, in important amounts, but that it is so firmly associated with tissue constituents that its presence is only revealed by extraction, when a chemical process is used which breaks that association. It may even be suggested that, in some cases of diabetes, the defect may be in the power of making insulin available from the pancreas, and distributing it in the blood to the tissues needing it, rather than in the loss of the power to produce it. Insulin may be formed in sufficient amount for minimal normal needs, but some condition may interfere with its secretion, destroy it on the way to the seat of its action, or in some way so antagonise its effect that a

diabetic condition results. Only by introducing the factor of availability can we yet explain such observations as the recorded extraction, from the pancreas of a patient dead of diabetic coma, of sufficient insulin to have kept him alive for a month, if artificially injected at the proper times.

It is evident that there are many facts still to be elucidated concerning the nature of insulin and its relation to the constituents of the various tissues. Such facts as are available do not to any degree weaken the evidence in favour of the pancreatic islets, as the place of its origin and the main site of its storage. We hardly need further evidence on that point than the fact that, in spite of the proved possibility of obtaining it—by which, of course, we can only mean something apparently acting like it—from many other organs, and in spite of the special difficulties which the pancreas itself presents, pancreas is still the only practicable material for its production, in the now world-wide and commercially important process of its manufacture.

With regard to the difficulties which prevented its earlier discovery, in addition to its sensitiveness to proteolytic enzymes, I have already mentioned the ease with which it is lost by adsorption on to protein precipitates. I should add that there is now good evidence that it is apt to be mixed with other substances, also extracted from pancreas, which are not merely inert, but definitely antagonistic to insulin in action. We do not know what these are, or what parts of the pancreas they come from, or whether

they have any physiological significance. But it is quite easy to obtain a preparation from pancreas exhibiting no insulin action, and even causing a rise of the blood-sugar percentage, from which, nevertheless, by careful fractionation, a good yield of insulin can still be obtained. I suspect that this masking of the insulin effect, by the presence of antagonistic substances, played a large part in the frustration of the earlier efforts to obtain the pancreatic hormone.

It will have been quite obvious to you that this varied information as to the properties and distribution of insulin could not have been obtained, if observers had been dependent for all their tests on the reaction of naturally diabetic patients, or of dogs rendered artificially diabetic by removal of the pancreas. Progress, indeed, was enormously accelerated by the discovery, made by the Toronto workers, that insulin not only brings the blood-sugar concentration of the diabetic subject back towards the normal level, but rapidly lowers that of the normal animal. The blood of a normal animal, such as a rabbit, which has been kept fasting for a day, usually contains from 90 to 120 milligrams of glucose in 100 c.c. When a sufficient dose of insulin has been injected this sugar promptly begins to disappear from the blood—immediately if the injection has been made intravenously, within fifteen minutes if it has been made hypodermically. By the end of an hour it may be as low as 50 milligrams per cent, by the end of an hour and a half 30 milligrams per cent—that is

to say, may have reached a level at which the traces of other reducing substances in the blood begin so to complicate the determination by any of the usual methods, that it would probably be more accurate to say that no significant amount of glucose is left in the blood. Before this condition is reached, and usually by the time the estimates, by one of the ordinary reduction methods, give a glucose reading of about 40–45 milligrams per cent, the animal, after premonitory symptoms of nervous excitability, falls into violent convulsions, often of a rotatory type in the rabbit. Analogous symptoms are seen in the human patient, and in some diabetic subjects may be experienced when the blood-sugar has fallen but little, if at all, below the level characteristic of normal human blood. The patient feels hungry, flushes and grows pale, sweats profusely, becomes tremulous and unsteady, and suffers from impairment of vision. In extreme cases unconsciousness and even convulsions have been produced. Whatever their exact meaning and origin, there can be no doubt that the symptoms are conditioned by the fall in the blood-sugar, since they are promptly removed by introducing more glucose into the blood. In the rabbit this is usually done by injection, while in human cases it is usually sufficient to give sugar by the mouth. Mann and Magath had previously observed similar symptoms to develop in dogs deprived by operation of the liver, as soon as the concentration of glucose in the blood fell below a certain value.

This effect on the blood-sugar of normal animals

provided a basis for the quantitative determination of insulin, without which no progress could have been made in determining its properties. The effect, on any one individual animal, is roughly proportional to the dose, when working with doses not too small to produce a definite effect, or so large as to cause convulsions from hypoglycæmia. Several methods of measurement have been used. Some workers avoid blood-sugar determinations and are content to observe the production of convulsions or of death in small animals, such as rats and mice. For such a test large numbers of animals must be used, owing to the wide individual variations of sensitiveness, and the assay depends on observing the proportion of animals thrown into convulsions, or alternatively killed, by a certain dose. Originally the Toronto school defined a " unit " of insulin as the quantity needed to produce convulsions in a fasting rabbit weighing 2 kilograms. This unit was found in practice to be too variable, and somewhat inconveniently large for clinical dosage. Later, therefore, a smaller unit was defined in terms of the average lowering of the blood-sugar, over a period of five hours after the injection. Macleod and Orr give the following formula:

$$\frac{a}{b} \times \frac{w}{v} \times 1{\cdot}5 = \text{units per c.c.}$$

where a = the difference between the blood-sugar percentage before the injection and the average percentage during the five hours following injection, b = the difference between the original percentage

of blood-sugar and 0·045 per cent, w = the weight of the rabbit in kilograms, and v = the volume of the solution injected in c.c.

It will be seen that this formula again attempts to give an absolute determination in terms of an animal reaction, and the wide variations of sensitiveness between different rabbits make its application difficult and uncertain in its results, in my experience, unless, again, very large numbers of animals are used for each test, which in this case would entail an enormous number of blood-sugar determinations. We have evidence, further, that apart from individual variations, there may be large systematic differences in the response of rabbits in different countries. In these circumstances I felt bound to advocate the redefinition of the unit in terms of a stable standard sample of insulin, for the preparation of which Dudley's method of obtaining insulin as a dry hydrochloride, through the picrate, appeared particularly suitable. The principle was accepted by an International Conference. A quantity of dry insulin hydrochloride amounting to about a quarter of a million units was prepared, and within the last few weeks, as the result of concordant tests made in Canada, in the United States, and in this country, the Toronto Committee have defined the unit of insulin as the activity contained in one-eighth of a milligram of this standard, dry powder. This gives the possibility, whatever method is used, of standardising the reaction of the animals which are employed for the test, and should ensure, in the near future,

that a unit of insulin means the same thing in all countries. In my own laboratory, a preparation to be assayed and the standard preparation are tested in succession on the same individual rabbits, even diurnal variations being eliminated as far as possible by testing on each day half the animals with the standard preparation, and half with the unknown sample, reversing the order on the two days (Marks).

LECTURE IV

THE PANCREAS AND INSULIN (*continued*)

In my previous lecture I spent some time on the meaning of a unit of insulin, because it is important, not merely from the point of view of practical therapeutics, but even from that of physiology, that the term should have a fairly precise and uniform significance. The question of main interest for physiology, however, and obviously one of the greatest importance for any theory as to the course of carbohydrate metabolism in the body as a whole, is that of the mechanism by which an excess of insulin, introduced into the circulation, causes the disappearance of glucose from the blood. This action, in one form or another, has provided the basis for the available methods for detecting and measuring insulin. The question is obviously related to, but by no means necessarily the direct converse of, the question why glucose should accumulate in the blood, and pass out into the urine, when the normal supply of insulin from the pancreas is absent or deficient. It was only to be expected that, as soon as insulin became available for experiment, the attention of workers in different countries would be directed to this fundamental problem of its action. The flood of literature on the subject has, indeed, been beyond any reasonable

expectation. A recent review of the publications, since Banting and Best's results were first made known about three years ago, gives references to nearly 600 papers. Naturally a certain proportion of these have tended to obscure rather than to clarify the issue. At the same time, I think we can begin to distinguish the direction from which the light is coming.

Let us begin by eliminating some of the simplest possibilities, which first suggest themselves. There is no trustworthy evidence that insulin has any effect upon glucose as such. Papers have appeared from time to time suggesting that glucose disappears when incubated with insulin in simple, watery solution. I feel convinced that Grevenstuk and Laqueur are justified, in regarding any positive results obtained in such experiments as significant only of the dirtiness of their authors' methods of working.

I think we may also take it as established, that insulin does not cause disappearance of glucose from the blood by acting on any constituent of the blood itself. There has been some conflict of evidence on this point, which really begins with an earlier discussion, as to whether in the shed blood of a diabetic patient, or of an artificially diabetic dog, glycolysis proceeds at a normal rate or not. Minkowski found that it did, Lépine that it was much reduced, others that it was abnormally rapid. The same kind of discrepancy is found with regard to the action of insulin, added to the blood *in vitro*, or injected into the animal before bleeding. The fact is that, even

in normal shed blood, glycolysis is a very variable phenomenon, depending on the preservation of the leucocytes, on the hydrogen ion concentration, and probably on other variable factors as well. I am quite convinced that, when precautions are taken to keep the conditions as constant as possible, and to exclude bacterial action, blood to which insulin is added shows no measurable increase in the rate of glycolysis, as compared with the same blood without insulin. Certainly we can discover along these lines no explanation for the rapid disappearance of glucose from the blood in the body.

The next possibility which presented itself for consideration was that the sugar lost from the blood was deposited as glycogen in the liver. That under certain conditions the disappearance of sugar from the blood under insulin may be associated with the appearance of glycogen in the liver, was early apparent from the observations of the Toronto workers, on dogs from which the pancreas had been removed. In such dogs, if untreated, it is known that the liver very soon loses its store of glycogen completely. When they were treated for a period with insulin and sugar, and then killed for examination, it was found that the liver had acquired a large load—up to 12 per cent of its weight—of glycogen. It was obvious that this glycogen was formed from glucose, which, in the absence of insulin, would have appeared as such in the blood, and have been lost in the urine. At the same time the respiratory quotient rose, indicating that some

sugar was being oxidised. We may take it as proven, then, that insulin can cause the disappearance of sugar, at least when excess of sugar is available, by promoting the oxidation of some and the deposition of some in the liver in the form of glycogen.

You must remember, however, that in treating the diabetic dog with insulin we are replacing the missing hormone, and thus enabling the animal to deal with sugar which would otherwise appear in the blood in enormous excess. When we study the cause of the disappearance of sugar from the blood of a normal animal, the conditions are different. We take an animal which already has a sufficient supply of insulin for its normal needs, usually we lower its carbohydrate reserve by withholding food for a period, and then we suddenly introduce into its circulation a large excess of insulin. The question was whether, under such conditions, insulin was also producing its whole effect by promoting oxidation of sugar and its deposition as glycogen in the liver. If this had been the case, the question of its action would have been a relatively simple one. Experiment soon showed that it was not so. You may remember my mention of Mann and Magath's experiments, in which they succeeded in removing the liver from a dog with so little hæmorrhage or shock that it was, for a time, relatively normal. I described how, as the sugar of the blood and tissues became used up, the blood-sugar gradually fell, and ultimately hypoglycæmic symptoms appeared. There was time before this, however, to test the effect of

insulin, and the result of its injection into the liverless dog was that the blood-sugar fell rapidly, as in the normal fasting animal. Clearly, then, the liver was not essential for the production of hypoglycæmia, though the possibility was still open that the sugar might be deposited as glycogen elsewhere, as in the muscles.

We may follow this question, of the organs necessary for the removal of sugar by insulin, through to its end. Olmsted and Logan found that the brain, or the whole head, could be removed without interfering with the insulin effect. In the spinal animal kept under artificial respiration insulin still produced a typical disappearance of blood-sugar. Meanwhile Hepburn and Latchford had shown that the isolated heart, perfused with Ringer's solution, removed glucose from the solution more rapidly when insulin was added. Finally Burn and I took the decapitated, spinal animal, removed the stomach, intestines, kidneys, and all the ductless glands, and excluded the liver from circulation. In this preparation, consisting only of the bones, muscles, and skin, with the heart to keep the circulation going, and the artificially ventilated lungs to aërate the blood, we balanced the use of sugar by a very slow, constant infusion of glucose, so that the blood-sugar kept a steady level. Then we injected insulin and found that the blood-sugar fell rapidly, unless we enormously increased the rate of infusion. Finally we skinned the preparation, and still got the same effect. It was clear, then, that the skeletal muscles, in addition to the liver,

were the important agents in the disappearance of sugar under insulin.

But what had become of the sugar? Had it been oxidised, or stored in the muscles as glycogen? Evidence of another kind had meanwhile been obtained, which made it very difficult to suppose so. Dudley and Marrian had taken mice, killed a batch of controls for analysis, and injected the others with insulin. As soon as the symptoms of hypoglycæmia, showing that the blood-sugar had disappeared, were seen in each of the insulin mice, it was killed and its tissues worked up. Analyses were made of the glycogen in the liver and in the muscles. So far from any increase being apparent, to account for the free sugar lost from the blood and tissues, the glycogen also had practically all disappeared, from liver and muscles alike. Clearly the lost sugar had not been converted into glycogen under these conditions; on the contrary, as the ready-formed sugar had been removed, it must be supposed that glycogen was hydrolysed to replace it, until at last the whole of the sugar originally present in the body, either as such or potentially as glycogen, had somehow disappeared. What could have become of it? Was it possible that it was all oxidised? Experiments carried out by Dudley and Laidlaw and by Trevan and Miss Boock seemed to give a negative answer again. They measured the oxygen absorbed and CO_2 expired by mice, first in a normal control period, and secondly in successive periods after injection of insulin, beginning the observations as

soon as the mice had returned to quiescence, after receiving the injection and being put back into the metabolism chamber. A rapid fall was recorded, both in the absorption of oxygen and the output of CO_2. The measurements of the two kinds were not made in the same experiments, so that a calculation of the respiratory quotient could not be made.

I felt some doubt at the time as to the interpretation of these results. It seemed to me just possible that the necessity of waiting, even for a short period, for quiescence to be re-established after the injection, might have excluded from observation the period in which the most rapid oxidation was taking place, and that, by the time the observations began again, the metabolism might already have been depressed by hypoglycæmia. In order to justify such an assumption, however, it would be necessary to suppose that the excessive dose of insulin produced a condition corresponding to the opposite extreme of that seen in diabetes. Instead of an impairment of the power to metabolise sugar, and a rapid formation of sugar from proteins and fats, as seen in diabetes, we should have to suppose that the whole of the oxidative metabolism was concentrated on the pre-existing carbohydrate, and the formation of sugar from other materials stopped. The same idea has been put forward by Laufberger, who suggested, indeed, that the entire action of insulin is thus to suppress the formation of sugar from other substances. The view that excess of insulin somehow concentrates metabolism on carbohydrates seems to be supported by

more recent evidence; but the idea that the disappearance of the carbohydrate might be entirely due to its oxidation is, in my opinion, no longer tenable. Nor is it possible to suppose that the depressed formation of sugar from proteins or fats is alone sufficient to explain the effects.

The view that the carbohydrate was really removed by oxidation, the increased oxidation of sugar being so balanced by the depressed metabolism of other substances that the total respiratory exchange was not materially increased at any stage, and even rapidly declined as the carbohydrate became used up, was put forward with much reserve in a lecture which I gave about two years ago. The idea received strong support from Lesser, who combined it with the view that the energy liberated by the oxidation of the sugar was at first used, in part, for the synthesis of a further quantity of sugar to glycogen. This conception he supported by a series of ingenious experiments with Bissinger and Zipf. It must be emphasised that they were working with doses of insulin too small ever to produce a pronounced hypoglycæmia, and injecting sugar at the same time. Their method was to inject the insulin and glucose into a series of mice, kill them at intervals, and analyse each animal for glycogen and sugar, without distinction of organs. Controls, injected with the same quantity of glucose alone, were treated in the same way. In the controls the sugar disappeared in about three hours and about half of it appeared as glycogen. In the insulin series the same kind of change took place, but occu-

pied only half an hour. The process of oxidation of glucose and the concurrent formation of glycogen were therefore accelerated by the insulin to about six times the normal rate.

Here we appear to be dealing with the same process as we already encountered in the effect of insulin on the diabetic dog. Very possibly it is, as Lesser suggested, an oxidative synthesis, analogous to the recovery process of muscular contractile metabolism, in which, as Meyerhof demonstrated, 2 molecules of lactic acid are oxidised, to provide energy for the re-synthesis of 6 molecules to glycogen. It is well to bear in mind that this may possibly be the chief function of insulin under physiological conditions, that is to say, when excess of sugar, in relation to the supply of insulin, is available for metabolism. We cannot, however, explain wholly on these lines the action of insulin when it is present in excess, the most familiar example being the effect of a relatively large dose on the normal animal. I have been forced to abandon the suggestion, made two years ago, that the whole of the carbohydrate lost under these conditions might have been oxidised, and Lesser, who had earlier supported this idea, has likewise come to regard it as inadequate. I shall not have time to review the whole of the evidence on the point, and shall have to be content with presenting that with which I am most familiar.

You will remember that I mentioned experiments by Hepburn and Latchford on the isolated and perfused heart of the rabbit, in which they showed

that glucose was removed from the perfusion fluid faster, when the latter contained insulin. Hepburn and Latchford could detect neither an increase in the heart rhythm nor a deposition of glycogen, to account for the glucose which disappeared. Burn and I repeated these observations and confirmed the acceleration of sugar loss by insulin. We measured, in addition, the CO_2 production. The heart was sometimes accelerated in the insulin period, and the CO_2 output accordingly increased. But the increased energy output of the heart, as measured either by the rate of its rhythm, or by the output of CO_2, was seldom sufficient to account for more than a part of the additional glucose which disappeared under insulin. The CO_2 output was often less than in the pre-insulin period, in spite of the accelerated loss of glucose. Meanwhile Plattner, in Starling's laboratory, had made experiments on the glucose removed from the blood of a heart-lung preparation, and had discovered no increased rate of removal when insulin was added, except such as might be accounted for by the accompanying acceleration of the beat. It may be that, under the more physiological conditions of working of the heart in the heart-lung preparation, the sugar taken up under insulin is rapidly carried to a further stage of metabolism, and oxidised to provide contractile energy. In any case, however, it was clear that the heart, the survival of which is associated with a rhythmic and not readily controllable mechanical activity, was not an ideal object for the settlement of this question. On the other hand, the

decapitated and eviscerated preparation, in which the heart served merely to maintain the circulation, and the carbohydrate metabolism was, for practical purposes, that of the quiescent skeletal muscles, was much better suited to our purpose, of discovering how much of the sugar which disappeared under insulin was oxidised. The rate of sugar disappearance was greatly accelerated by the injection of insulin, even when the supply of glucose by intravenous infusion was kept constant. When the latter was increased as soon as the insulin was injected, in the endeavour to prevent the blood-sugar concentration from falling, the acceleration of sugar disappearance was relatively enormous.

Under such conditions, and, indeed, in any case when glucose was constantly supplied, some increase in the rate of oxygen absorption was always observed to follow the injection of insulin ; but this increase was only sufficient to account for a relatively trivial proportion of the glucose which disappeared.

The method enabled us to test some further possibilities as to the fate of the glucose. The respiratory quotient of this preparation, with the blood-sugar artificially maintained by infusion, was always practically unity, indicating that the metabolism of the resting muscles was purely that of carbohydrate. It may be noted that the recent work of Meyerhof and of A. V. Hill and his colleagues strongly suggests that the contractile energy of muscle is similarly obtained from carbohydrate alone, and that, when other foodstuffs are used, these must

be converted into glucose elsewhere—presumably in the liver—and presented in that form to the muscles. However that may be, the respiratory quotient of this preparation showed that, under the conditions of our experiment, the muscles were burning sugar only. If any significant part of the glucose removed under insulin had been directly converted into fat, this transformation must have revealed itself by a rise of the quotient to well above unity. A rise above unity should also have followed if any significant part of the glucose had been merely cloven into lactic acid, as some have suggested. No such rise in the respiratory quotient was ever detected.

Now in these experiments, since only carbohydrate was being metabolised in the period before insulin, we cannot possibly explain the effect of the latter by a transfer to the carbohydrate of metabolism which was previously using fat or protein. A merely oxidative removal of sugar at an increased rate under insulin would be bound to reveal itself by an acceleration of oxidation, proportionate to the accelerated disappearance of glucose. We have seen that, though some acceleration of oxidation was detected, it was of relatively very small dimensions. The conclusion is inevitable, that the main bulk of the glucose which disappears, when insulin is present in excess, has undergone a non-oxidative transformation.

The later experiments of Lesser, in which he measured the respiratory exchange in mice injected with sugar only, and in those injected with the same amount of sugar and varying doses of insulin,

lead to a similar conclusion. The respiratory quotient rose immediately when insulin and sugar were given together, showing that a proportion of the fat and protein previously metabolised was being spared by the direct oxidation of sugar ; the rise in the quotient only occurred after a long interval when sugar alone was given. But the amount of sugar lost by oxidation, as calculated from the respiratory data, was always less than the loss of sugar found by direct analysis of the mice for sugar and glycogen ; and the discrepancy was greater, the larger the dose of insulin given. Lesser's figures fully confirm the finding of Dudley, Laidlaw, Trevan, and Boock, that when insulin is given in doses which eventually produce obvious symptoms, its action is attended throughout by a progressive depression of the oxidative metabolism.

Obviously the next step required is the discovery of the form in which the missing glucose has thus been removed, beyond the reach of the ordinary methods of detection. On this point we have still no clear evidence, but only hints and suggestions. Probably the most significant is the observation, first made by Wigglesworth and others, that, as glucose disappears from the blood, the concentration of inorganic phosphate in the blood shows a decline. The phosphoric acid hexose-esters have played such an important part during recent years in the unravelling of the carbohydrate metabolism, from Harden and Young's work on the fermentation of sugar by yeast, down to that of Emden and of Meyerhof on the carbohydrate metabolism of muscular

contraction, that it was natural, and almost inevitable, that attention should turn in this direction, in seeking for the missing sugar. I do not think, however, that the evidence yet available warrants the attempt to give this suggestion greater precision. It is tempting, of course, to suggest that the steps intervening between glucose and glycogen, in the process for dealing with a carbohydrate excess, are similar to those figuring in the known re-synthesis of glycogen from lactic acid in the recovery process of muscular contraction, and that the lost sugar is laid down in the form of the so-called lactacidogen. Such evidence as we have, however, does not give support to such an idea. Dudley and Marrian found, and Kuhn and Baur have confirmed the finding, that the muscle of an animal, killed during extreme hypoglycæmia produced by insulin, not only contains less pre-formed lactic acid than a normal muscle, but elaborates less during post-mortem incubation. Nor is there any reason to conclude that insulin intervenes in the contractile metabolism of muscle at all. This process seems not to be abnormal in muscle from an animal without a pancreas, and not to be affected directly by insulin. The evidence we have points rather to insulin being concerned in the storage of carbohydrate not needed for immediate use. Probably the lost sugar is held in some intermediate stage of synthetic elaboration, and possibly in a complex containing phosphoric acid.

As to its further fate, however, we can only speculate. It may be that the complex represents a stage

on the way between sugar and glycogen, and that the synthesis is, for some unknown reason, arrested at this intermediate equilibrium-point when excess of insulin is available ; it may be, on the other hand, that it has nothing to do with the glycogen synthesis, and represents a stage on another metabolic path—e.g. that from sugar to fat. If the hypoglycæmia is evanescent, and the animal recovers, some of the reappearing glucose may come from a breakdown of the unknown temporary complex ; though I think the evidence is, on the whole, unfavourable to such a supposition.

There is one other set of observations on which I must touch before leaving this section of the subject, though their meaning is by no means clear.

Winter and Smith estimated the reducing power of a protein-free filtrate from normal blood, and compared this with the rotatory action on polarised light. They found that the rotation was much lower than what would be expected, if the reducing action were due to glucose in the ordinary equilibrium form, which it takes on in watery solution—the so-called α-β-glucose. If the purified and concentrated filtrate, on which the rotation was determined, was kept for some days, the dextro-rotatory power gradually increased, until it corresponded with that of an α-β-glucose solution, of the concentration which the copper reduction had indicated. Having observed, further, that the fresh filtrate would reduce a permanganate solution in the cold, they concluded that the glucose of normal blood exists in the unstable,

highly reactive γ-form. They thought that, by a change in its rotatory power, they could detect the conversion of ordinary α-β-glucose into γ-glucose, when it was incubated with insulin and liver extract. In blood from diabetic patients, on the other hand, the protein-free filtrate showed a *higher* dextro-rotatory power than an α-β-glucose solution of the same copper-reducing power, and they took this to indicate that the blood contained α-β-glucose and a more complex sugar in addition. According to their view, then, the function of insulin is to convert ordinary glucose into the reactive γ-form, in which form alone the body can utilise it. The theory, and the evidence on which it was based, have been vigorously criticised ; and, indeed, the conclusion that normal blood-sugar is glucose in the γ-form is clearly not justified by the polarimetric discrepancy, observed in such a complex solution as the blood filtrate. On the other hand, the latest evidence, obtained with ultra-filtrates from normal and diabetic bloods, confirms the suggestion that there is a difference in their relative rotatory and reducing powers, which needs explanation. It does seem likely that, when insulin is present, some constituent appears in the mixture of different forms of glucose, which we call the blood-glucose, which lowers the dextro-rotation, and which may represent a stage preliminary to removal from the blood as glycogen, or as the unknown complex ; but we shall do well to be careful not to go beyond the evidence in drawing conclusions as to its nature.

From all this evidence, and much more, which at first sight may easily give the impression of confusion and conflict, I think we may safely extract the following conclusions, as reasonably well established by the evidence:

1. When excess of glucose is present, insulin causes an increase in the rate of its oxidation, and of its synthesis to glycogen.

2. When insulin is present in excess, a large proportion of the glucose which disappears is neither oxidised nor synthesised to glycogen, but changed into some form in which it escapes detection by available chemical means, and is withdrawn from oxidative metabolism.

3. The fact that, while carbohydrate is thus being withdrawn, the respiratory quotient nevertheless rises, suggests that the formation of new sugar from fat and carbohydrate is simultaneously depressed.

While recognising that what is known as "insulin" exhibits these various actions, it must be borne in mind that no approach has been made to the separation of a pure substance. More than one principle may be involved, but in default of evidence for more than one, we continue to speak of "insulin" as having this complex action.

The problem of the disappearing sugar is not in reality a new one. Long before the discovery and separation of insulin, it had been shown that, when glucose is injected into an animal, and the total is added of what can be discovered in the body as unchanged glucose and as glycogen, what has been

excreted in the urine, and what, according to the showing of the respiratory metabolism, has been oxidised, there remains an important balance still to be accounted for. We shall see that there is reason to suppose that the normal secretion of insulin is concerned in producing this discrepancy also. It cannot be doubted that the discrepancy is the same as that which is encountered in analysing the effects of insulin given by artificial injection. In the latter case, however, when insulin is present in great excess of the metabolic need, the discrepancy is accentuated, and becomes so great as to dominate the picture.

We have reached now, by a natural transition, the normal function of insulin in the body, and the evidence for its being a true hormone. Let us consider first the reaction of the normal organism to the sudden introduction of an excess of carbohydrate. Most of you will be familiar with the carbohydrate tolerance test, based on a determination of the blood-sugar at intervals during an hour or two after swallowing 50 grams or so of glucose. In a normal subject the blood-sugar rises from the fasting level, which is in the neighbourhood of 100 milligrams per cent, to a maximum of 150 or more, which is reached somewhat about the end of the first hour after taking the glucose. It then falls again, and by the end of the second hour is frequently well below the fasting level, reaching 70 milligrams, or even less, in some cases. It then gradually returns to the normal again. In the diabetic subject the blood-sugar not only rises to a much higher percentage

when sugar is given, but remains high for hours, and only slowly falls again towards the initial level.

The point to which I ask your special notice is the fall of the blood-sugar below the normal level, in the normal person, as a sequel to the alimentary rise. There is very good reason for attributing this fall to an accelerated output of insulin. I shall mention some rather striking evidence on that point presently in another connection. For the present I may refer to some observations of Spiro and of Stähelin, who showed that blood taken from a normal person a few hours after a meal, at the time of the post-alimentary fall, and transfused into a diabetic subject, caused a prompt and decided fall in the abnormal blood-sugar percentage of the latter. Blood taken from the same normal individual, when his blood-sugar was below the average level as the result of a fast, had practically none of this effect.

Let us further glance very hastily—for I must not allow myself to be drawn into a detailed discussion of an enormous subject, with which I have little familiarity—at the nature of the defect in human or in artificial diabetes. We have mentioned three actions of insulin, without committing ourselves to any conclusion as to whether they really represent different functions, or are parts of a single complex. All are absent or defective in diabetes. (1) Insulin promotes oxidation of glucose and synthesis of glycogen ; in diabetes both are defective. On the other hand, there is no reason to suppose that the use of glucose in the contractile metabolism of muscle

is influenced by insulin, or defective in the diabetic. (2) Insulin removes some sugar by converting it into an unknown complex; this function, too, is in abeyance in the complete diabetic. A dog without a pancreas, if given carbohydrate in its diet, excretes it quantitatively as sugar in its urine. (3) Finally, insulin appears to depress the formation of sugar from fat and carbohydrate; and the unrestrained and wasting progress of this conversion is one of the conspicuous features of natural or of artificial diabetes, and is very probably responsible for other metabolic anomalies which characterise it, such as the ketosis.

We can, I think, feel that we have good evidence for the function of insulin as a true hormone, and for the rise of blood-sugar as the effective stimulus to its secretion. Regarding the secretion as not unvarying, but as fluctuating with the call made on the pancreas by the blood-sugar, if not actually intermittent, we easily reach the probability that the occurrence of ketosis in prolonged fasting, or on a diet of pure fat, or as the result of continued vomiting, may be due to the lack of the normal stimulus to secretion of insulin, and the consequent disorder of metabolism, seen also in diabetes, when its production is in constant defect. The prompt removal of ketosis by administering glucose receives, similarly, a more satisfactory explanation.

We have now to consider the still very incomplete, but rapidly growing body of evidence, as to the relation between the action of the pancreas and its internal secretion and that of other ductless glands.

Two in particular have been shown to have an action which is directly or indirectly antagonistic to that of insulin. The Toronto workers early showed that the hypoglycæmia produced by insulin, and the symptoms accompanying it, could be relieved by an injection of adrenaline. Many efforts have since been made to demonstrate a direct antagonism between these two hormones, but, according to my reading of the evidence, they have failed. The suggestion early arose that, since adrenaline enriches the blood with sugar by stimulating the liver to hydrolysis of its glycogen store, insulin should hinder this effect, and prevent the depletion of the liver glycogen by adrenaline. Most attempts to demonstrate such an antagonism, by perfusion experiments on livers, have failed completely ; indeed, the most striking result of such perfusion experiments has been their general failure to demonstrate that insulin affects the glycogen store of the liver in either direction, by increasing the storage or accelerating the loss. On the other hand, experiments on the whole animal have shown very clearly that, when the liver holds a large glycogen deposit, the removal of sugar from the blood under insulin is rapidly compensated by its output from the liver ; and there is direct evidence that an accelerated output of adrenaline is concerned in this conversion of the liver glycogen (Houssay). Whether insulin directly acts on the suprarenal gland, or whether this latter is sensitive to a fall, as the pancreas is to a rise of blood-sugar, we do not know. In either case there

is no good ground for suggesting that the two hormones directly antagonise one another. The evidence points rather to the action of adrenaline being to produce a compensatory output of sugar from the liver, when the level in the blood begins to fall as the result of the action of insulin.

The action of the pituitary extract is rather more obscure. The action of the extract by itself, on the blood-sugar of the normal animal, is comparatively small ; a slight hyperglycæmia is the usual effect of its injection. Its action in checking the production of hypoglycæmia by insulin is proportionately very large (Burn). The evidence would seem to point to a more direct antagonism in this case. Such a conception is in harmony with what we know of the carbohydrate tolerance in cases of pituitary defect. Large quantities of sugar can be taken in this condition, without producing a rise of the blood-sugar sufficient to lead to escape into the urine. We may suppose that the normal antagonism of the pituitary hormone, modifying the effect of the pancreatic insulin, is in abeyance. The insulin then exercises its effect, in preventing hyperglycæmia, free from this normal check.

Lastly, I must say something about some experiments, giving very striking and instructive results, which are still incomplete, and which bring the action of the thyroid gland into the picture. Removing the thyroid gland from a rabbit greatly increases the sensitiveness of its response to insulin. Conversely, the first effect of administering thyroid substance is greatly to increase the resistance to insulin (Burn and

Marks). There is no doubt, I think, that these effects are due to modification of the compensatory output of sugar from the liver. This is under control of the sympathetic nerves and of adrenaline, and the natural interpretation of the effect of the thyroid is to suppose that its secretion increases the sensitiveness of the liver's response to its natural, sympathetic stimulus. The first effect of thyroid feeding, then, while the liver still holds a good glycogen store, is to produce so rapid a compensatory output of glucose that the effect of insulin is nipped in the bud. Simultaneously with this fall of sensitiveness to insulin, it can be shown directly that the sensitiveness to adrenaline—the hy*per*glycæmia produced by a given dose—is greatly increased. The effect has been, therefore, to shift the issue of the contest, between removal of sugar by insulin and new formation excited by adrenaline, in favour of the adrenaline effect. After more prolonged thyroid feeding, however, another state of the balance is produced. The over-sensitive response of the liver has emptied its glycogen store, till it is incapable of further response. The normal check being thus rendered ineffective, the animal becomes more sensitive than ever to the artificial injection of insulin. Not only so; the response to a natural gush of insulin from the pancreas, unopposed by the compensating output of sugar from the liver, may be astounding in its dimensions. Under such conditions an actual injection of glucose may, as the result of the pancreatic response to the sudden, artificial hyperglycæmia, be followed by a secondary

fall of the blood-sugar, so rapid and to so low a level, that the animal dies in hypoglycæmic convulsions. Similarly, an injection of adrenaline, given when the liver has been almost emptied of glycogen by thyroid feeding, may cause the last remnants to be thrown rapidly into the blood stream as sugar, and by so doing evoke an excessive gush of insulin from the pancreas, from the uncompensated action of which a fatal hypoglycæmia may again result (Marks).

There are points in this complex of antagonisms still needing explanation. It may be that thyroid feeding causes an over-sensitive response of the endocrine mechanism of the pancreas as well as of the liver, and that excessive insulin-production is compensated and overshadowed by excessive glycogenolysis, until exhaustion of the liver allows it to become manifest. I have said enough, however, to indicate what a delicate balance of opponent and compensatory hormone effects must be involved in the normal metabolism of carbohydrates, a central and all-important part being played by the pancreas, with its delicately responsive mechanism for the production of insulin in proportion to the need for its action.

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Four Lectures
By J. C. Drummond

TITLE OF LECTURES

LECTURE

I. Modern Views on the Mechanisms of Biological Oxidations

II. Modern Views on the Mechanisms of Biological Oxidations (*continued*)

III. Certain Aspects of the Rôle of Phosphates in the Cell

IV. The Vitamins

LECTURE I

MODERN VIEWS ON THE MECHANISMS OF BIOLOGICAL OXIDATIONS

If observation be made of the oxygen taken up by the unfertilised egg, it will be found that the oxidative processes measured in this manner, and if necessary controlled by estimation of the output of carbon dioxide or liberation of heat, are initially of a low order of magnitude. Even this degree of cellular activity does not last, but progressively diminishes until the cell ultimately dies: and it may be noted that the falling off of oxygen utilisation by the egg under these conditions runs, in an approximate manner, parallel to the gradual resolution and final disappearance of certain stainable units of the cell structure. A similar parallel, and one which holds for us even more interest, may be traced in the case of the fertilised ovum. It does not matter whether the initiation of the developmental processes is caused by entry of the sperm, or whether it results from exposure of the egg to chemical or physico-chemical conditions that will induce artificial parthenogenesis, for in both cases the formation of the development membrane is accompanied by an almost

explosive outburst of oxidative activity. At this stage the respiration rate may, according to Shearer, increase nearly eightyfold within the first few minutes.

Our belief in the association between structure and oxidation in the cell is, however, supported by more convincing evidence than that which has just been described. Warburg has provided at least two striking examples from his studies on cellular respiration. In the first place, he has observed that the oxygen utilisation of the nucleated red-blood cells of birds is greater than that of human red corpuscles, and that the younger nucleated cells consume more than the older ones, in which the proportion of stainable structures is smaller. Furthermore, the respiration of the corpuscles does not fall to any great extent if the blood is carefully hæmolysed, leaving the nuclei intact, the important part played by these units being apparent from the fact that they show the characteristic oxygen consumption after removal by centrifugalisation, but fail to do so when their internal structure has been injured by disintegration. Even more striking are his experiments on fertilised sea-urchin eggs which were showing the greatly increased respiratory activity which follows fertilisation. If these eggs were subjected to grinding, so that the newly formed structures were destroyed, the oxygen utilisation promptly fell to the level shown by the unfertilised egg and then continued to decrease slowly to zero level.

In both cases, that of the unfertile egg and that of

the developing cell injured by mechanical means, the relatively small amount of oxidative activity is definitely associated with the presence of granules, which continue to show the same degree of activity after they have been separated from the main bulk of the egg by centrifugal force. Granted, therefore, that there are adequate experimental observations to justify our associating oxidation in the cells with certain visible structures, it is necessary to inquire how far this view can be reconciled with our present knowledge regarding the actual chemical or physico-chemical mechanisms of oxidative processes in the cell.

In the first place, it must be obvious that it is not at variance with our current views on the nature of enzyme action by which so many oxidations are known to be effected. Whatever may be the actual chemical constitution of these remarkable synthetic and analytic agents of the living cell, there can no longer be reasonable doubt that their activities, and probably, to some extent, even their specific characters, are more dependent on the nature of their active surface than on what may be termed their gross chemical composition. It would be superfluous, therefore, to emphasise in this lecture that oxidations that are carried out in the living cell by the agency of the thermolabile enzymes are catalytic reactions in heterogeneous systems.

Up to a short time ago the view was somewhat generally held that all biological oxidations are catalysed by enzymes of one type or another, but the

researches of Hopkins and of Meyerhof have, during the last year or two, demonstrated the existence of oxidation-reduction systems which may operate efficiently without the participation of any unit of the nature of an enzyme. How far these newly discovered mechanisms will be found to be dependent for their efficiency on the presence of certain structural surfaces is as yet uncertain, but the most recent work strongly suggests that such units are in most cases an essential part of the system.

The phenomenon of narcosis is essentially an inhibition of the oxidative processes in the cell. It will be recalled that independently two investigators, Meyer and Overton, attempted to explain the phenomenon by showing that the power of a substance to induce narcosis was related to its partition coefficient in an oil-water mixture. In other words, the greater its relative solubility in the oil phase, the more potent it would be to induce narcosis. Attractive as their theory at first appeared, it was not long before many cases were pointed out (e.g. the urethanes) in which narcotic action stood in no relation to the ratio of solubility in oil and water. A more satisfactory theory was some time later advanced by Traube, in which he related the narcotic action to the power to lower surface tension. As regards covering the case of the urethanes, or the very important case of hydrocyanic acid and its salts, his theory was little or no better than that of Meyer and Overton, but the recent work of Warburg has shown us that his views were a big advance on theirs

when explaining the narcotic action of many organic substances, such as the series of alcohols.

We may conveniently deal in the first place with one of Warburg's studies on a biological reaction. The fermentation of sugar solutions by the cell-free press-juice of yeast is inhibited by narcotics just as are the oxidative processes of the living cell. The inhibition caused by the members of a homologous series of alcohols runs parallel with their power to cause precipitation of protein in the solution, and it can be shown that removal of the enzyme in this manner is caused by the changes at the surface of the protein particles rendering them more sensitive to salt precipitation. Still more striking are the experiments which Warburg has made with his so-called charcoal model. He has found that a few typical oxidations in the body can be imitated by allowing certain substances in aqueous solution to react with atmospheric oxygen at the surface of blood charcoal that has been carefully prepared. In this manner the amino-acids, glycine and cystine, are rapidly oxidised to simple products, carbon dioxide, ammonia, and sulphates, although normally they are practically unaffected by exposure to air or oxygen. The parallel with the cell is, however, much more striking when we learn that such oxidations are also inhibited by narcotics. The charcoal model should provide us, therefore, with a valuable means of studying the dynamics of certain types of oxidations in the cell, if we admit the relationship, or in any case of observing a most interesting type of simple

oxidation at a surface. No one could have made better use of these opportunities than the discoverer himself.

In the first place, he has proved beyond reasonable doubt that the oxidation of the α-amino-acid in aqueous solution takes place after adsorption on the surface of the charcoal particles has occurred, and that inhibition by narcotics such as the alcohols is due to interference with this preliminary process by changes in surface tension. Warburg imagines the narcotic actually displacing the amino-acid molecules from the surface of the carbon, and with this picture provides an adequate explanation of his experimental observations. But we are still faced with the case of the narcotics, particularly the cyanides, which cannot be explained in the same manner, and for which we must seek some more satisfactory explanation of their inhibitive action.

The Rôle of Iron in Oxidations

Warburg discovered that the activity of his model was not always of the same magnitude, and that if the charcoal was prepared from a very pure material, such, for example, as highly purified cane sugar, cyanides would exert no inhibitory action. A number of experiments were made with samples of charcoal containing different amounts of iron, and from the results it was inferred that the smaller the

percentage of iron the charcoal contained the less marked is the anti-catalytic action of hydrocyanic acid. The presence of traces of soluble iron salts in the solution in which the amino-acid and charcoal were brought together did not lead to a retardation of oxidation by cyanides, but a powerful inhibition was brought about when the iron was distributed in a fine state of division throughout the charcoal by adding iron salts to the pure organic substance before carbonising. Very highly active materials were also prepared by careful charring of the iron-rich pigment hæmatin. One result of this work was to add even more evidence to the large amount already existing to show that traces of iron exert a very marked catalytic activity on biological oxidations, a point to which we shall again refer, but another was that it led Warburg to form the opinion that the inhibition of oxidation caused by cyanides is due not to changes in surface forces such as cause the reacting substances to be displaced from the charcoal and replaced by a narcotic alcohol, but to a loose chemical combination with the iron present as one unit in the surface structure of the granule.

Let us now examine the main steps by which Warburg imagines a simple oxidation to occur at the surface of a granule of charcoal rich in iron. Undoubtedly he is right in believing that adsorption of the substance occurs, probably as a monomolecular layer spread over the whole surface of the particle.

The view that the reacting substance is spread

over the surface of the catalyst in a monomolecular film is based on the well-known observations of Langmuir and others on adsorption. It is one that is in better agreement with data regarding the velocity of reactions in heterogeneous systems than that of Bodenstein and his supporters, who believe that the speed of such changes is determined by the rate of diffusion of the reacting substances through the layer of end-product formed on the surface of the catalyst. The mere adsorption at the surface is naturally insufficient to initiate oxidation, which is dependent upon the presentation of oxygen in a suitable form.

Later in our discussion we shall be dealing with the question of "activation" of oxygen, that is, the process by which it is believed that the relatively inactive molecular oxygen O_2 is converted into the active atomic form. For the present it may be said that Warburg regards the iron-rich areas on the surface of his charcoal particle as being the sites at which activation of oxygen occurs.

Inhibition of the oxidative processes may therefore be brought about either by physical changes, such as the alterations in surface tension which cause a narcotic alcohol to replace the substrate at the active surface of the particle, or by chemical means when an inactivation of the essential iron units is caused by some form of combination with the toxic agent, as in the case of cyanides.

There can be no question that Warburg has presented us with a conception that is in general agree-

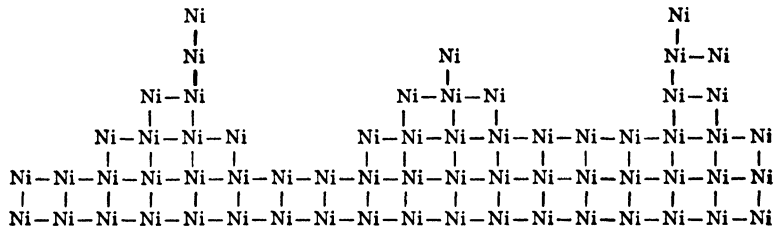
ment with our existing knowledge regarding surface catalysis, and it is particularly interesting in this connection to note that his studies of the oxidation of leucine at the iron-charcoal surface led him to support the opinion that only a small proportion of the total surface of the catalyst can be regarded as active in promoting oxidation. This view is confirmed by the differences noted by him between the adsorptive powers of charcoals and their action as catalysts of oxidation, and is one that is finding wide acceptance amongst those studying catalysis in heterogeneous systems, not only in the field of laboratory research, but also in the wide sphere of technical catalysis.

The appreciation of the fact that not by any means the whole of the surface of the charcoal particle is capable of catalysing oxidations has led Warburg to portray such surfaces as mosaics of areas rich in iron and poor in iron, the former being more active agents in promoting oxidations.

It is probable, however, that a more satisfactory conception of the structure of the surface of such particles is being gained from the careful studies of Rideal in this country and of Scott Taylor in America.

To explain the inequality of catalytic power exhibited by adjacent areas on the surface of a catalyst such as the nickel particles used so widely for industrial reductions, or, as they are termed, hydrogenations, the latter investigator has recently advanced an ingenious suggestion which may be

illustrated, although only in two dimensions, by the sketch.



This diagram is intended to represent the arrangement of the atoms of nickel at the surface of a catalytically active granule. Those in the mass of the particle are regularly arranged in the symmetrical lattice structure revealed by X-ray analysis, but at points on the surface there may occur projections of atoms that have not yet adopted the fully symmetrical arrangement. Such atoms, particularly those represented as being at the tips of the projecting peaks, cannot be regarded as having the same degrees of constraint as those in the mass, and might be regarded as existing in a state intermediate between the metallic and the gaseous. The affinities of such atoms can be regarded as important factors governing reactions occurring at their surface. The importance of this hypothesis lies in the fact that it gives us a graphic picture of the catalytic metal granule which in general appears to satisfy the great mass of observations regarding catalysis at such surfaces, and in particular explains the loss of activity caused by heat or poisons, as well as the variations which may occur in the proportion of the total surface of a granule which is catalytically active. It

is of interest to note that in 1922 Armstrong and Hilditch expressed the view that " an active catalyst " is merely an average term expressing a surface in which a number of patches of maximum activity occur, the greater part of the surface being of a quite low order of activity. The theory is important to us in our consideration of the Warburg model, because it provides a working hypothesis that explains not only the existence of the more highly active iron-containing patches, but also the loose type of combination with cyanides which results in inhibition of oxidation.

It is obvious that, whatever the nature of the reaction between iron and cyanides in the carbon model may be, it is a reversible one in the living cell, provided the doses of the poison are sub-lethal. Lovatt Evans, amongst others, has drawn attention to this point, and more recently the researches of Starling and Verney on the action of cyanides on the secretion of urine by the isolated kidney have provided ample confirmation of the fact.

Now Taylor's hypothesis is quite consistent with a reversible reaction involving a loose compound of iron and cyanides, and Prof. Warburg informed me recently that he had been able to satisfy himself of the reversibility of the inactivation of the catalytic action of iron by hydrocyanic acid in a simple example of catalysis in a homogeneous system. The oxidation of oxalic acid to carbon dioxide and water under the action of iodates proceeds very slowly under ordinary conditions, but is greatly accelerated in the presence

of a trace of iron. Addition of hydrocyanic acid in low concentrations at once inhibits the reaction, which is, however, resumed if the toxic agent is removed by aeration. Whilst it is certain that these facts stand in direct relation to the inhibition of oxidation by cyanides in the body, they do not help us to form a mental picture of the actual state of the iron present in living structures. We can rest content to leave this problem to the future, and to turn to the more urgent question of the actual rôle played by the iron. As we have already remarked, Warburg would have us believe that it effects an activation of oxygen, thereby rendering it capable of vigorous oxidative action.

Activation of Oxygen

The fact that oxygen present in the atmosphere is a relatively inert substance was recognised as far back as 1840 when Schonbein, from his careful studies of ozone and hydrogen peroxide, formed the opinion that some form of activation takes place before actual oxidation. From that time on we have had a long succession of theories regarding the nature of this so-called activation, but few of these merit serious attention to-day. Of the more important views, we may mention the hypothesis of van't Hoff, according to which molecular oxygen is activated by a kind of ionisation,

$$O_2 \rightleftharpoons O + O$$

and that of Hoppe Seyler, which, derived from his

studies on anaerobic fermentations, regarded nascent hydrogen as the activating agent:

$$O_2 + H \cdot\cdot H \rightarrow H_2O + O$$
$$X + O \rightarrow XO$$

These two theories paved the way for the more important one advanced by Traube, which laid the foundation of our modern views regarding the rôle of peroxides in oxidations. His work is primarily important for the emphasis which he laid on the part played by water in a large number of oxidative changes.

The oxidation of hydrogen at a palladium surface was visualised by him in the following manner:

$$\begin{matrix} PdH_2 \\ PdH_2 \end{matrix} + \begin{matrix} HO \cdot\cdot H \\ HO \cdot\cdot H \end{matrix} + O_2 = 2Pd + 2H_2O + H_2O_2$$

oxidation of a second substance, X, present, occurring by the action of the hydrogen peroxide:

$$X + H_2O_2 \rightarrow XO + H_2O$$

At one time the main objection to the views of Traube were based on failure to recognise the intermediate formation of peroxide in such oxidations, but, as we shall see, this difficulty has been considerably lessened by more recent work.

The theory of Traube was developed along br lines by Bach and by Engler, both of whom fav the idea of an intermediate formation of com of the peroxide type, the best illustrative being that of the well-known autoxidation

aldehyde, alone or in the presence of indigo, which may be represented by the equations,

$$C_6H_5\cdot CHO + O_2 \rightarrow \underset{\text{benzaldehyde superoxide}}{C_6H_5\cdot CO\cdot O\cdot OH}$$

$$C_6H_5\cdot CO\cdot O\cdot OH + C_6H_5\cdot CHO \rightarrow 2C_6H_5\cdot COOH$$

or in the presence of indigo which becomes oxidised rapidly to isatin,

$$2C_6H_5\cdot CO\cdot O\cdot OH + \underset{\text{indigo}}{C_{16}H_{10}N_2O_2} = 2C_6H_5\cdot COOH + \underset{\text{isatin}}{2C_8H_5NO_2}$$

The reactions may be written in the general manner:

$$\begin{aligned} A + O_2 &\rightarrow AO_2 \\ AO_2 + A &\rightarrow 2AO \\ \text{or } AO_2 + B &\rightarrow AO + BO \end{aligned}$$

Where A is a substance capable of reacting with molecular oxygen to form a peroxide, whilst B is a substance ordinarily stable to molecular oxygen, but readily oxidised by the peroxide formed by A. From our point of view the importance of this peroxide theory, as it may be termed, is that it was applied by Bach and Chodat with considerable success to account for the action of the so-called oxidases, the heat-sensitive agents discovered originally by Schonbein, and studied later with great care by Bertrand and other investigators.

Oxidases

Regarded the oxidase system as consisting of the components, each inactive in the absence her member. One he term[1] oxygenase,

which he believed to be a thermolabile substance capable of uniting with molecular oxygen to form a peroxide, that is, substance A in the above equation, and the second an agent (peroxidase) capable of catalysing the breakdown of this compound with liberation of active oxygen capable of bringing about the oxidation of another substance (B).

It is curious to note how similar to his and Traube's views were those held by Schonbein about fifty years previously, for the latter observer believed in the existence of thermolabile " Sauerstofferreger " which, entering into a loose combination with ozone, formed peroxides capable of giving off oxygen in an activated state for the oxidation of other substances. The study of these complex systems has been carried many steps further by the work of Onslow, who has, I believe, satisfactorily demonstrated that the substance which takes up molecular oxygen is not, as Bach suggests, itself an enzyme, but an autoxidisable cell unit—she favours the idea that it may be related to catechol—which forms a peroxide under the influence of an enzyme, oxygenase.

It is, however, doubtful whether one explanation will serve to cover all the many types of oxidation brought about by the agency of the oxidases. Gallagher has presented evidence that the autoxidisable substance, which he believes may be of the nature of a lipin, may require no oxygenase to accelerate the formation of the peroxide, and that the only enzyme required is the peroxidase to decompose this intermediate compound.

Somewhat similar views are expressed in a recent paper by Thurlow, in which she points out that formation of an intermediate peroxide would satisfactorily account for certain facts concerning the xanthine oxidase. Her work, as well as that described in the later section dealing with glutathione, raises the question whether the peroxide formed is in all cases an organic peroxide or whether hydrogen peroxide may not sometimes be formed. On chemical grounds she doubts the intermediate formation of a xanthine peroxide, and leans to the view that the enzyme causes dehydrogenation of the purine with formation of hydrogen peroxide.

$$\text{xanthine} + \text{xanthine oxidase} \rightarrow \text{uric acid} + 2H$$
$$2H + O_2 \rightarrow H_2O_2$$

Support to this view is given by the observation that in the presence of nitrates the reaction may result in their reduction to nitrites, the former substances acting as acceptors of the hydrogen.

The xanthine oxidase would appear to be the agent described under the name of atite by Haas and Hill, as being present in milk and possessing the power to reduce nitrates to nitrites.

In the same connection we can refer to the well-known Schardinger enzyme present in fresh milk, which causes the rapid decolorisation of methylene-blue in the presence of an aldehyde, because it is obviously a closely related complex (see later).

The intermediate formation of organic peroxides in certain biological reactions must be regarded as

proven, and there is little doubt that the enzymes termed the peroxidases are the agents that break down such compounds and liberate oxygen in a form capable of carrying out vigorous oxidation of substances otherwise unaffected or only slowly attacked by molecular oxygen. It is of interest to observe, by way of linking up this section with an earlier one, that in his laborious efforts to purify preparations of peroxidase and gain a knowledge of its chemical nature, Willstätter has confirmed the view that iron is concerned in the action of this agent. From horse-radish he prepared by a tedious series of fractional adsorptions a product containing 60 per cent of the activity, but weighing rather less than one ten-thousandth part of the original mass of root tissue. The activity of any one preparation was at each stage of its purification related to the amount of iron present, but that of different preparations was not always proportional to their content of that element.

Catalytic Effect of Metals other than Iron

Bertrand was the first to point out in his careful studies of the enzyme, laccase, responsible for the oxidation of the complex acid present in the sap of the trees from which lac is obtained, that its activity runs parallel with the amount of manganese present.

A similar relationship has also been traced between the presence of this element and the activity of the

enzymes that form the black and brown pigment known as melanins by the oxidation of tyrosine and possibly also other closely related phenolic substances. A glimpse of the part played by the metal in such cases is given us by the observations of Trillat and of Dony Hénault, which showed that careful precipitation of manganous hydroxide in the presence of a protective colloid such as albumin or gelatin yields a product showing striking resemblance to certain natural oxidases. Such synthetic oxidases will cause the blueing of guaiacum, will oxidise hydroquinone, and are destroyed by exposure to conditions which coagulate or otherwise change the colloidal system.

The catalytic effect of traces of copper has been noted by Meyerhof in connection with the autoxidations that occur in the presence of compounds containing the sulphydryl group, to which reference will be made later, so that it is not improbable that in lower animals, in which copper is found instead of iron as the metallic unit of the respiratory pigment, this element serves as a catalyst in a manner similar to the rôle of iron in more highly evolved species.

Dehydrogenation

In discussing Miss Thurlow's views on the mechanism of action of xanthine oxidase reference was made to her suggestion that the action of the enzyme was essentially a dehydrogenation of the xanthine molecule with subsequent formation of hydrogen

peroxide by the molecular oxygen serving as an *acceptor* of the activated or nascent hydrogen.

It is to Wieland that we turn for detailed exposition of the theory of dehydrogenation. He holds the view that oxidations in which there is direct taking up of molecular oxygen are comparatively few in number, being almost limited to the examples of direct peroxide formation, and that by far the great majority of oxidations are essentially dehydrogenations. Examples of his types of reactions may be given as follows :

1. Oxidative dehydrogenation (if atomic oxygen is available) :

$$R - CH_2 - OH + O \rightarrow R - C\begin{matrix}=O\\ \diagdown H\end{matrix} + H_2O$$

2. Autoxidative dehydrogenation :

$$RH_2 + O_2 \rightarrow R + H_2O_2$$

3. Oxidation by a hydrogen acceptor other than oxygen :

$$RH_2 + X \rightarrow R + XH_2$$

Where X is a substance, termed the hydrogen acceptor, having a marked affinity for active hydrogen. In reactions of types 1 and 2 oxygen can be regarded as the hydrogen acceptor.

In other words, Wieland thinks in terms of activated hydrogen atoms, whereas Warburg pictures an activation of the oxygen. Curiously enough, there has been a somewhat vigorous controversy between

the two schools of thought without its having been sufficiently appreciated that both processes doubtless occur.

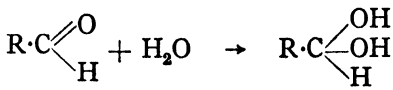
In passing, it might be mentioned that no more striking parallel could be traced between the activation of hydrogen and oxygen at non-living and at living surfaces than by comparing the remarkable similarity between the combination of these gases at the surface of spongy platinum and that which is effected by the remarkable bacteria described by Kasserer.

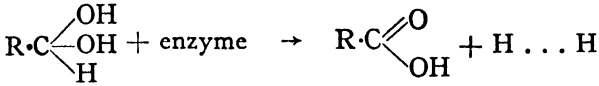
These extraordinary organisms share with the nitrite bacteria and certain of the so-called sulphur bacteria the power to build up their complex cell constituents from purely inorganic materials, carbon dioxide, nitrates, phosphates, etc., by means of energy derived from simple exothermic reactions, such as the direct oxidation of hydrogen, ammonia, or sulphur. It can well be imagined that such organisms resemble the primitive forms of life that existed at an extremely remote period in the history of life on the earth, and that they are, relatively speaking, not far removed from the inorganic colloidal systems from which they have slowly evolved.

To return to our main subject, it may be stated that the important part played by the so-called hydrogen acceptors is now generally admitted, so that a few illustrations may be of interest.

The Schardinger enzyme present in milk, to which allusion has already been made, can be regarded as

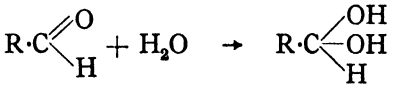
catalysing the oxidation of the aldehyde as set out in the following scheme:

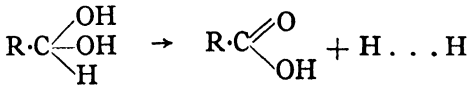


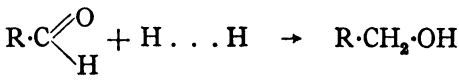


Methylene-blue + H . . . H → leuco-base of methylene-blue.

The well-known Cannizarro reaction provides a very similar example, in which a second molecule of aldehyde serves as the hydrogen acceptor, becoming thereby reduced to alcohol.







This reaction actually occurs in the animal body under the catalytic influence of an enzyme described by Parnas and by Battelli and Stern in 1910.

Many other examples might be given, but time will only permit reference to one extremely interesting case.

The experiments of Thunberg have shown that muscle tissue contains a thermolabile agent capable under anaerobic conditions of causing reduction of methylene-blue in the presence of certain substances,

of which succinic acid may be taken as an example. The reaction has been shown to be due to the formation of fumaric acid and hydrogen, the latter being

$$COOH{\cdot}CH_2{\cdot}CH_2{\cdot}COOH \rightarrow COOH{\cdot}CH{:}CH{\cdot}COOH + H \ldots H$$

accepted by the methylene-blue with consequent disappearance of its blue colour. The importance of this work is considerable, for it has been found to throw light upon several aspects of the breakdown of organic substances in the bodies of higher animals. In particular, it bears on the degradation of the straight-chain fatty acids, which from the classic work of Knoop was believed to proceed by oxidation at the β-carbon atom. In this manner the molecular chain could be broken down in stages, losing at each step two carbon atoms in the form of acetic acid. The work of Thunberg, as well as that of Battelli and Stern, suggests that the mechanism of this process is probably:

$$\begin{aligned}
R{\cdot}CH_2{\cdot}CH_2{\cdot}COOH &\rightarrow R{\cdot}CH{:}CH{\cdot}COOH + {}_2H \\
R{\cdot}CH{:}CH{\cdot}COOH + H_2O &\rightarrow R{\cdot}CHOH{\cdot}CH_2{\cdot}COOH \\
R{\cdot}CH{\cdot}OH{\cdot}CH_2{\cdot}COOH &\rightarrow R{\cdot}CO{\cdot}CH_2{\cdot}COOH + {}_2H \\
R{\cdot}CO{\cdot}CH_2{\cdot}COOH + H_2O &\rightarrow R{\cdot}COOH + CH_3{\cdot}COOH
\end{aligned}$$

Prof. Raper has informed me privately that he has recently obtained evidence that oxidation of the carbon·chain of fatty acids may occur at the α- and γ-carbon atoms as well as at the β-position. If this should be so, the existence of a system in muscle capable of dealing with succinic acid discovered by Thunberg and Battelli and Stern is of even more significance.

Thunberg is a hearty supporter of Wieland's views on dehydrogenation, particularly when substances of biological importance, such as the sugars, amino-acids, and fats are being considered, because, with the probable exception of the unsaturated fatty acids, no member of these foodstuffs appears to be liable to direct attack by molecular oxygen. His own words are "Tiefer gesehen ist Wasserstoff der gemeinsame Brennstoff der Zellen." On the other hand, Thunberg is amongst those who appreciate that activation of oxygen as well as hydrogen must occur, for he has observed that the reduction of methylene-blue by succinic acid *in vacuo* is not inhibited by cyanides, whereas in the presence of the muscle tissue and atmospheric oxygen the acceptance of hydrogen by the latter substance is prevented by the poison. He suggests that cyanide does not prevent hydrogen activation and transport, but that it prevents activation of oxygen. It is to be remembered that equilibria data have yet to be considered in respect to the empirical use of oxidising agents such as methylene-blue as substitutes for O_2. The work of Mansfield Clark should make clear their biological significance.

Hydrogen Peroxide in Biological Oxidation

It is interesting to trace how, from time to time, beginning with Schonbein in 1840, theories of oxidation involving the intermediate formation of hydrogen peroxide have been advanced. At one time there were objections to the idea that any part could be

played by hydrogen peroxide in cellular activity on the grounds that it is not only a toxic substance, but one that could not exist in living tissues owing to its immediate destruction by the universally distributed specific enzyme catalase.

It is, however, a highly significant fact, based mainly on Dakin's researches, that many substances yield on oxidation with hydrogen peroxide *in vitro* products similar to those which can be detected when they are oxidised in the living cell. To give only a few examples, we may take the case of benzene oxidised to phenol, catechol, and quinol, both in the animal body and by hydrogen peroxide in the test-tube, similarly the oxidation of indole to indoxyl, of glucose to glycuronic acid, and of butyric acid to β-hydroxybutyric and acetoacetic acids. Knowing what one does of the products which are formed by the action of other oxidising agents one cannot but be impressed by the parallel which Dakin has traced. If we accept the theories of dehydrogenation that we have just been considering, we must recognise it as highly probable that hydrogen peroxide arises from the acceptance of hydrogen by molecular oxygen; after all, oxygen must, from Wieland's point of view, be regarded as normally the chief hydrogen acceptor in the body. The decomposition of the resulting hydrogen peroxide will occur by the agency of the enzyme catalase, and will liberate atomic oxygen for direct oxidations in the immediate locality.

It may, therefore, be accepted both from the

purely chemical studies of Dakin, and from the exhaustive investigations of tissue oxidations by the other workers we have mentioned, that oxidations by the agency of hydrogen peroxide do occur in the living cell. Again a link may be made between this stage of our study and an earlier section by recalling the discovery by Fenton of the catalytic effect which small traces of iron have on oxidations *in vitro* by means of hydrogen peroxide.

LECTURE II

MODERN VIEWS ON THE MECHANISMS OF BIOLOGICAL OXIDATIONS (*continued*)

THE PART PLAYED BY WATER IN BIOLOGICAL OXIDATIONS

As already pointed out in dealing with the question of the activation of oxygen, it is to Traube that we are indebted for first pointing out the important part played by water in oxidations. The essential principle advanced in his theory is, as you all know, generally accepted throughout chemical circles to-day. From the biochemical aspect we owe much to Bach for his developing the idea into a conception of biological hydrolytic-oxidation reduction systems. It is only necessary briefly to indicate these ideas of the participation of water in oxidations in the cell by one or two characteristic examples.

One of these may be taken from a preceding lecture and illustrates the probable rôle of water in the degradation of straight-chain fatty acids in the living cell.

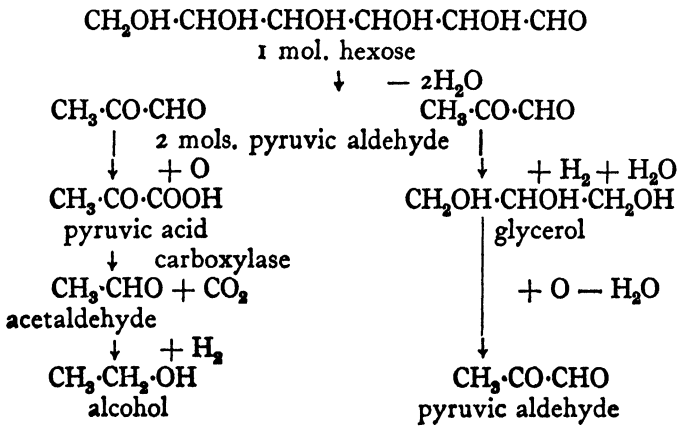
The essential steps by which degradation of the carbon chain is effected by successive removal of pairs of carbon atoms are, as we have seen, probably:

$$\begin{aligned}
R\cdot CH_2\cdot CH_2\cdot COOH &\rightarrow R\cdot CH{:}CH\cdot COOH + 2H \\
R\cdot CH{:}CH\cdot COOH + H_2O &\rightarrow R\cdot CHOH\cdot CH_2\cdot COOH \\
R\cdot CHOH\cdot CH_2\cdot COOH &\rightarrow R\cdot CO\cdot CH_2\cdot COOH + 2H \\
R\cdot CO\cdot CH_2\cdot COOH + H_2O &\rightarrow R\cdot COOH + CH_3\cdot COOH
\end{aligned}$$

In such an oxidation there normally occurs definite utilisation of oxygen to accept the hydrogen removed from the acid molecule in stages by the catalytic agent (" hydrogen transportase ") present in the cell. Another type of oxidation may, however, occur when degradation of such organic substances occurs under anaerobic conditions, for in such cases another form of hydrogen acceptor is essential for the reaction to proceed. Many of the oxidations studied by Battelli and Stern, or by Thunberg, in which methylene-blue is added to replace the missing oxygen as a hydrogen acceptor, might be given as examples, but an even more striking one may be taken from a natural process. The fermentation of sugar by yeast to alcohol, carbon dioxide, and traces of other organic substances is one of the chemical reactions known to man from earliest times. The mechanism of the change has, however, only recently been explained by the careful researches of Harden in this country, Fernbach in France, and C. Neuberg in Germany. One of the main clues to the whole series of changes which appear to take place during the breakdown of sugar by zymase was obtained when C. Neuberg showed that by carrying out the fermentation in the presence of sulphites the main products formed were not alcohol and carbon dioxide, but glycerol and acetaldehyde, both of which had previously been detected in small amounts in normal fermentations. The relation between the amount of sulphite added and the yield of acetaldehyde and glycerol is well shown by the following figures, from which it is

Sodium Sulphite.	Cane Sugar.	Acetaldehyde.	Glycol.
33 gms. . .	100	11·90	23·37
50 „ . .	100	12·52	24·86
75 „ . .	100	13·89	27·61
100 „ . .	100	18·65	36·90

apparent that side-tracking the acetaldehyde by making it form a non-reacting compound with sulphite inhibits the production of alcohol and carbon dioxide and increases the yield of glycerol. In passing, it may be remarked that this discovery was actually turned to practical advantage in Germany during the war, when considerable quantities of glycerol for explosives were prepared from the relatively plentiful carbohydrates with an equivalent economy of the scarcer fats. The normal progress of the fermentation of sugar is now believed to occur according to the scheme given in broad outline below, in which the initial stages of fermentation, in which the sugar forms a phosphate, later to be broken down by the enzyme hexosephosphatase, are not represented as they do not directly concern us here. They will, however, be referred to in a later lecture.



Now, let it be noted that almost quantitative degradation of the sugar to alcohol and carbon dioxide occurs without the intervention of atmospheric oxygen. All oxidations involved in this scheme can be regarded as effected by the agency of water and are, therefore, accompanied by reductions of other substances which act as appropriate hydrogen acceptors. Two such oxidation-reduction stages appear to be present, and leave us with 1 molecule of sugar giving rise to 1 molecule each of carbon dioxide, alcohol, and pyruvic aldehyde.

The latter substance is, however, itself a stage in the production of alcohol and CO_2, and must, therefore, in due course, be practically all converted into these final end-products. If, however, the reduction of the acetaldehyde to alcohol be prevented by removing the former substance from the sphere of reaction by combination with sulphite, then the parallel oxidation of glycerol to pyruvic aldehyde cannot occur, and glycerol will necessarily accumulate. It is necessary to point out that the sulphite compounds of pyruvic aldehyde and acid are fermentable.

Glutathione

It can be readily demonstrated that the respiration of muscle tissue or yeast cells practically ceases after they have been repeatedly extracted with water to remove soluble constituents. A more surprising fact is, however, that on replacing the extracted substances respiration will be resumed. Prof. Otto Meyerhof

in investigating this remarkable fact discovered that the ability of the aqueous extracts to restore the oxidation processes shows a certain parallel with their power to give a characteristic coloration with sodium nitro-prusside. This reaction had been previously studied by Mörner (1901), Heffter (1908), and Arnold (1911), from whose work it had seemed probable that the substance responsible for the colour reaction is either free cysteine, $CH_2 \cdot SH \cdot CH \cdot NH_2 \cdot COOH$, or another relatively simple molecule containing the sulphydryl group (—SH).

Of these investigators, Heffter clearly appreciated that such a substance could function in the tissues as an agent in oxidation-reduction reactions, possibly, as he suggested, in the following manner:

$$2\,R-SH + O_2 \rightarrow R-S-S-R + H_2O_2$$
$$X + H_2O_2 \rightarrow XO + H_2O$$

(X being an oxidisable substance)

He also indicated the possible rôle of such a disulphide compound as a hydrogen acceptor by referring to the reduction of cysteine by sodium sulphite.

$$\begin{array}{l} CH_2-S-S-CH_2 \\ \quad | \qquad\quad | \\ CH\cdot NH_2 \qquad CH\cdot NH_2 \\ \quad | \qquad\quad | \\ COOH \qquad\quad COOH \end{array} + Na_2SO_3 + H_2O \rightarrow 2 \begin{array}{l} CH_2-SH \\ | \\ CH\cdot NH_2 \\ | \\ COOH \end{array} + Na_2SO_4$$

It was clearly indicated, therefore, that Meyerhof should attempt to replace the "Atmungskörper" or respiratory substance present in the aqueous extracts of muscle and yeast by a pure substance of the type

of cysteine. This he did, but without any marked success in restoring the oxidative activity of the cells. He was more successful, however, when he tested the simple compounds thioglycollic acid, $\begin{matrix} CH_2SH \\ | \\ COOH \end{matrix}$, and thiolactic acid, $\begin{matrix} CH_3 \\ | \\ CH - SH \\ | \\ COOH \end{matrix}$, because in these cases he observed considerably larger amounts of oxygen were absorbed than would have been necessary merely to oxidise the added acids to the disulphide forms:

$$\begin{matrix} CH_2 - S - S - CH_2 \\ | \qquad\qquad | \\ COOH \qquad COOH \end{matrix} \quad \text{and} \quad \begin{matrix} CH_3 \qquad\qquad CH_3 \\ | \qquad\qquad | \\ CH - S - S - CH \\ | \qquad\qquad | \\ COOH \qquad COOH \end{matrix}$$

Significant as his results undoubtedly were, they did not fully explain the action of the natural yeast or muscle extracts, because neither thiolactic nor thioglycollic acid had ever been detected as a constituent of living cells. Accordingly the isolation in pure condition by Prof. (now Sir) Gowland Hopkins in 1921 of a simple substance present in tissues and containing the sulphydryl group marked a very great advance.

This substance he termed glutathione, and, as he showed, it is a dipeptide of glutaminic acid and cysteine. Its constitution, as determined both by

analysis and by synthesis in Hopkins's laboratory, is represented by the formula:

$$\begin{array}{l} NH\cdot CH(CH_2\cdot SH)\cdot CO_2H \\ | \\ CO\cdot CH_2\cdot CH_2\cdot CH(NH_2)CO_2H \end{array}$$

It is, of course, as all such compounds are, capable of being converted by oxidation into the disulphide form; this reversible change being conveniently represented thus:

$$2\,G-SH \rightleftharpoons G-S-S-G$$

In the light of Meyerhof's studies it was not surprising that the discovery of glutathione should be followed by the demonstration that the addition of this substance to the washed yeast or muscle tissue restores the power of the cells to effect oxidations, as represented by oxygen utilisation, carbon dioxide liberation, or the decolorisation (i.e. reduction) of added methylene-blue.

Furthermore, it is possible to show that tissues possess the means not only of converting the sulphydryl form into the disulphide form, but of effecting the reverse change. Evidently, therefore, in glutathione we have a compound that can act not only in effecting hydrogen transport but also in oxygen transport, but before passing on to consider these processes in more detail, it will be best if we first draw attention to a remarkable fact noted both by Meyerhof and Hopkins.

The discovery of the power of thioglycollic and thiolactic acids and glutathione to restore the oxidation

processes in washed muscle tissue was in itself remarkable, but even more striking was the revelation that respiration is shown on the addition of these sulphydryl compounds to washed cellular material that has been "killed" by treatment with acetone or by being heated to 100° C.

In the light of these facts, it is no longer possible to view biological oxidations solely as reactions catalysed by agents (enzymes) sensitive to heat, and it is obvious that the oxidative capacity of the washed and boiled muscle tissue supplemented by the sulphydryl compound must be due to a system which differs essentially in character from the oxidases, or the thermolabile agents so extensively investigated by Bach, Thunberg, and Battelli and Stern.

The reduction of oxidised glutathione to the sulphydryl form by the heated tissue residues indicates that there is a hydrogen donator in these residues that is relatively insoluble in water, so that we can regard the respiration of this heat-stable system as occurring by some such process as the following equation indicates:

$$XH_2 + G - S - S - G \rightarrow 2\,GSH + X$$

(XH_2 representing the thermo-stable hydrogen donator in the tissues)

the associated oxidation of the reduced glutathione involving transport of atmospheric oxygen.

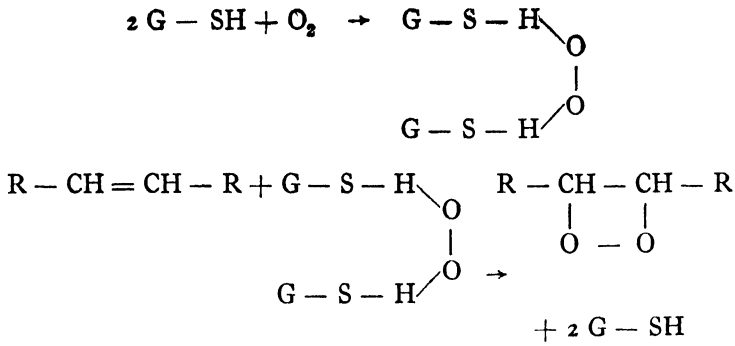
$$2\,G - SH + O_2 \rightarrow G - S - S - G + H_2O_2$$
$$XH_2 + H_2O_2 \rightarrow X + 2H_2O$$

This reaction will only cease when the supply of oxidisable constituent XH_2 is exhausted.

It is obvious, therefore, that the cell, for all living tissues yet examined have been found to give the nitroprusside reaction indicating substances with the –SH grouping, possesses, in addition to the relatively delicate systems represented by the oxidising enzymes, other more stable systems in which certain substances in close association with structural elements are oxidised, aerobically or anærobically, by the participation of sulphur groups of the type found in glutathione. At once questions will arise in one's mind as to the nature of the tissue constituent represented in the above equations by XH_2.

Meyerhof has ascertained that the part played in this relatively simple oxidation system by the washed and heated muscle tissue can to a certain extent be replaced by the material precipitated by acetone from the alcohol-ether extract of the tissue. Such material would, from its method of preparation, be largely of the nature of lecithin, and a reduction in the degree of unsaturation which occurs during the oxidation at once attracts attention to the highly unsaturated acids, such as linolenic acid, which may form part of the lecithin molecule.

Work from Hopkins's laboratory also points in this direction, for it has there been found that the absorption of oxygen by emulsion of an oil containing highly unsaturated fatty acids (e.g. linseed or cod-liver oil) is greatly increased by the presence of the reduced form of glutathione, which acts as a transporter of oxygen possibly, as Hopkins thinks, by the intermediate formation of a loose type of peroxide:



an alternative scheme:

$$R-CH=CH-R + HOH + G-S-S-G \rightarrow R-CHOH-CHOH-R + 2\,G-SH$$

being that favoured by Meyerhof, but rendered less acceptable by the fact that the oxidised form of the dipeptide is itself unable to cause an acceleration of the oxidation of such oils. The addition of the tissue factor, possessing as it does the power to reduce the disulphide, will have that effect.

These facts suggest that a compound such as linolenic acid is not identical with the tissue constituent which has the power to reduce the disulphide. Probably, therefore, the system represented by the heated and washed muscle tissue to which glutathione has been added may be represented by the following series of reactions which will proceed until the supply of the hydrogen donator X is exhausted.

$$2\,G-SH + O_2 \rightarrow G-S-S-G + H_2O_2$$

$$R-CH=CH-R + H_2O_2 \rightarrow R-\underset{\displaystyle OH}{\underset{|}{CH}}-\underset{\displaystyle OH}{\underset{|}{CH}}-R$$

$$XH_2 + G-S-S-G \rightarrow 2\,G-SH + X$$

The nature of the tissue constituent X is therefore still uncertain.

The oxidation of the sulphydryl group of glutathione which occurs in the presence of methylene-blue as a hydrogen acceptor has been examined by Dixon and Tunnicliffe, with the result that it is found to be an autocatalytic reaction accelerated by the disulphide formed.

Of great importance in considering the glutathione system is the study of the conditions under which it is most efficient. In his original paper Hopkins emphasised the important control exerted by the hydrogen-ion reaction of the medium. On the alkaline side of neutrality the acceleration of the reduction of methylene-blue by the disulphide form of glutathione in the presence of washed muscle tissue was very much more marked than when the reaction was acid, and Hopkins suggested, by way of explanation, that the transference of hydrogen did not occur in acid media, so that the disulphide form of the dipeptide was merely competing with the methylene-blue as a hydrogen acceptor.

Actually the optimum reaction for the oxidation of reduced glutathione or cysteine is that of the body fluids, pH 7·4, a fact of obvious physiological significance.

There is another condition profoundly affecting the reversible changes that characterise the glutathione molecule, and, curiously, it is one that we have already referred to several times, namely, the presence of traces of metals, particularly iron.

Matthews and Walker in 1909 drew attention to the catalytic action of minute traces of iron in the autoxidation of cysteine, and Warburg has in all essentials confirmed their observations. In a recent communication from Hopkins's laboratory, Harrison has provided reliable evidence that traces of iron (10^{-4} mg.) cause a marked acceleration in the autoxidation of glutathione. Meyerhof has also noted a similar catalytic action excited by traces of copper in the autoxidation of thioglycollic acid.

General Considerations

In these two lectures an attempt has been made to present to you the more outstanding discoveries and opinions in the field of modern research in biological oxidations. The inadequacy of our treatment of so wide a subject is a matter for regret, but time did not permit a fuller study. By way of conclusion, a brief review of the main points is presented to your notice.

In the first place, we have gained the impression throughout our study that oxidations in the cell are, to a large extent, if not entirely, examples of catalysis in heterogeneous systems. There can be no question that in such cases the seat of reaction lies in the monomolecular films demonstrated by Langmuir and others, and which are adsorbed either on the surface of delicate colloidal systems, the so-called enzymes, or on other and relatively more stable surfaces, such as those Meyerhof and Hopkins have described.

Here the reactions would seem to occur, and to be accelerated to a marked extent by the presence of traces of certain metals in a highly active form. It is possible that Scott Taylor's conception of the condition of the active atoms on the surface of a catalytic particle of nickel may help us in the future to form a picture of the condition of the atoms of iron, manganese, or copper, which exert so powerful a catalytic action in the living cell, but as yet it only helps us to understand more clearly the mechanism of oxidation at the surface of Warburg's charcoal model.

Turning next to the oxidising enzymes, we find that our new conceptions of oxidation-reduction systems has enabled us to effect a very welcome weeding out of superfluous terms. Only a few years ago the oxidases, reductases, and so-called aldehyde-mutases, were regarded as distinct groups of enzymes, bearing little or no relation one to the other.

Now we appreciate that the same enzyme may be effecting an oxidation by the withdrawal of two atoms of hydrogen from an organic compound such as an aldehyde, and that these hydrogen atoms may be accepted by oxygen, methylene-blue, nitrates, or by another molecule of the aldehyde, to mention only a few hydrogen acceptors. Thus the one enzyme acting under different conditions is bringing about the changes formerly ascribed to (1) an oxidase, (2) a reductase, or (3) an aldehyde-mutase.

This welcome simplification of the overcrowded nomenclature of the enzymes will, I think, definitely bring us nearer the solution of the difficult problem

of ascertaining what lies behind the specificity of these bodies, because it keeps foremost in our minds the importance of determining in detail how far the action of one enzyme may be controlled by conditions. A striking example of how seldom such problems are viewed from the right angle is provided by the fact that only within the last year has the reversibility of the action of pepsin and trypsin, almost the first enzymes to be recognised, been satisfactorily demonstrated by Wasteneys by means of a few simple, clear-cut experiments.

Perhaps the most outstanding fact we have learnt from our review is that the cell possesses oxidation-reduction systems stable to temperatures which inactivate the enzymes. One unit in these systems, glutathione, is now recognised as a substance of simple constitution which can be synthesised in the laboratory, and is, as we have seen, a molecule which can assist both in transport of oxygen and of hydrogen in the cell.

LECTURE III

CERTAIN ASPECTS OF THE RÔLE OF PHOSPHATES IN THE CELL

To the biochemist phosphoric acid occupies a unique position by virtue of the fact that it occurs in the organism not only in the form of simple salts with the alkaline or alkaline-earth elements, but also in organic combination as compounds belonging to each of the three great classes of protoplasmic constituents, the proteins, fats, and carbohydrates. This remarkable fact, one imagines, denotes that phosphoric acid played a fundamentally important rôle during the period of the earth's history when living organisms followed the evolution of the organic from the inorganic.

Interest in the many forms in which phosphoric acid may appear as cell constituents has been marked ever since physiological chemistry attracted attention, but a few years ago it was, indeed, disappointing to review the knowledge of the subject and to realise how little was really known regarding these compounds and their significance in the living organism. Welcome relief came when the classic researches of Sörensen revealed the part played by the simple salts of phosphoric acid in the regulation of tissue neutrality, and since that time the progress of

physical chemistry and the employment of improved technique and of more exact methods in biochemistry have led to many important advances in our knowledge of the various phosphorus compounds found in animal and plant tissues, and their functions.

It is obvious that in the space of one lecture it would be quite impossible to attempt to review so large a field as this subject now presents, and much as I would have liked to describe how McLean, Rosenheim, and Levene have by their painstaking labours at last brought order into the chemistry of the phosphatides, how the structure of the nucleic acids has now been determined with a degree of certainty that at one time appeared impossible of attainment, how we are slowly learning from the work of Bloor and of Meigs the part played in the transport and metabolism of fats by those units that contain phosphoric acid, and how a hundred and one other advances of the first order of importance have been made within the last ten or fifteen years, I have reluctantly been forced, if I am to present any sort of ordered survey, to restrict my remarks to one small corner of this wide field.

I have decided to present to your notice some striking results that have followed from recent research on the compounds which exist between certain carbohydrates and phosphoric acid. I have chosen this subject because not only are the advances that have been made very far-reaching in their importance, but also because it appears to me that it will serve to connect in a rather slight manner the

two lectures on the Mechanism of Oxidations with that which follows on the Vitamins.

Actually the first compound of the type of a carbohydrate phosphate to be isolated from naturally occurring products was the substance phytin, obtained in a more or less pure condition from *sinapis niger* by Palladin in 1893. Schultze and Winterstein demonstrated some three or four years later that it yields on full hydrolysis phosphoric acid and the cyclic hexahydric alcohol inositol. The wide distribution of this curious substance in plant tissues, particularly in storage organs, such as grains, tubers, rhizomes, and bulbs, strongly suggests that it is of importance during development, but in spite of the fact that it was discovered so long ago, and that it has been the subject of a large amount of study, we are still completely without knowledge regarding its significance.

Phosphates in the Fermentation of Sugars by Yeast

The first clear indication that phosphoric acid by its combination with sugars plays an important part in certain reactions carried out by the cell was provided by the classic researches of Harden and Young some twenty years ago. It will be remembered that they observed that the addition of soluble phosphates caused a great acceleration of the fermentation of glucose, fructose, or mannose by the yeast enzymes, and that on investigation they found that a quantita-

tive relation existed between the amount of added phosphate and the increased production of carbon dioxide and alcohol.

These observations were followed up by the isolation of a compound which proved to be a diphosphate of a hexose sugar, and they were able to present the following equation as indicating the alcoholic fermentation of a hexose.

$$2C_6H_{12}O_6 + 2R_2HPO_4 = C_6H_{10}O_4(R_2PO_4)_2 + 2CO_2 + 2C_2H_5OH + 2H_2O$$

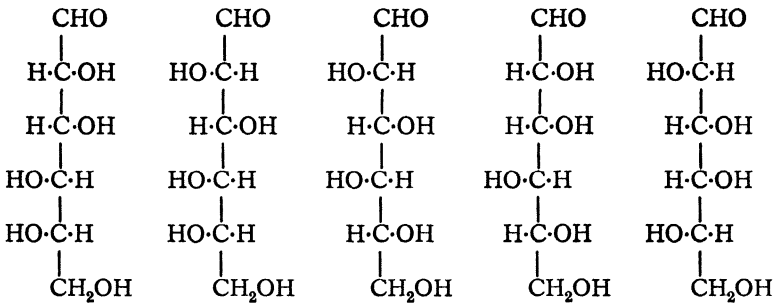
The further development of their work clearly demonstrated that yeast juice possesses the power not only to synthesise but also to hydrolyse the hexosephosphate by means of an enzyme, hexosephosphatase, and that it is apparently necessary that all the sugar pass through the stage of being combined with phosphoric acid before it is degraded by the steps described on p. 120. One is, therefore, naturally interested to enquire why the sugar should undergo this preparatory change, and one's first thought is to imagine that the sugar molecule is in some manner modified by passage through the intermediate phosphate so that its structure is more open to attack by the catalytic agents of the yeast. Such an idea is all very well in theory, but we must critically examine it from the experimental standpoint to see whether it is supported by any weight of evidence.

In the first place, it is reasonable to expect that the form of sugar liberated from the hexosephosphate on hydrolysis by the yeast juice might be the hypothetical modified form, and it is therefore of interest

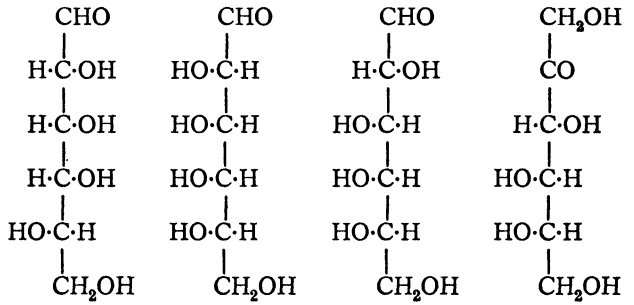
to ascertain what sugar has been isolated from this or a similar reaction. In passing it may be said that, as far as can be determined, the same hexosephosphate is obtained from fermentation mixtures whether glucose, fructose, or mannose is used ; these being the three fermentable hexoses. On decomposition of the phosphate Harden and his co-worker obtained a solution containing fructose and a small proportion of other sugars. This was confirmed by Neuberg, who actually isolated a considerable proportion of pure crystalline fructose, and who was inclined to believe that the only sugar yielded by the phosphate was fructose, and that the other sugars detected by Harden were products arising by secondary reactions ; it being well known that the sugars in faintly alkaline reaction undergo considerable molecular changes.

The idea that fructose might be the sugar moiety of the hexosephosphate molecule is supported by the certain knowledge that this hexose is more rapidly fermented than either glucose or mannose, and that when added to a fermenting solution of the other sugars it causes an immediate acceleration not shown by them. For the time being, therefore, it has been provisionally accepted. It also stands in relation to the interesting fact that the three fermentable hexoses share a common enolic form (formula X) which is not possessed by the other members. (See opposite page.)

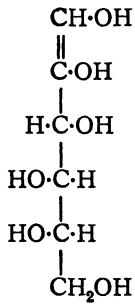
It is, of course, possible that a more labile form of hexose is actually present in the hexosephosphate, and that on hydrolysis in the normal course of



I. Mannose. II. Glucose. III. Idose. IV. Gulose. V. Galactose.



VI. Talose. VII. Allose. VIII. Altrose. IX. Fructose.



X. Common enolic form shared by I, II, and IX.

fermentation it is immediately broken down on being liberated, whereas in the absence of the agents which resolve the molecule it passes into the more stable sugar fructose. To solve this question it might be sufficient to apply Irvine's methylation

technique directly to the hexosephosphate, thereby "fixing" the sugar as it actually occurs in the complex and giving a reasonable chance of isolating it without secondary changes occurring.

The conception that sugars must pass through an intermediate stage of combination with phosphoric acid before they are broken down by the cell is not restricted to the narrow field of fermentation chemistry. Indeed, it would indicate a very narrow outlook if biochemists did not realise that, on an evolutionary basis alone, so fundamental a process as the degradation of sugars must in its essential steps be the same in plants and animals.

Phosphates in Relation to the Oxidation of Sugar in the Animal Organism

It is, therefore, not surprising that Embden was led some ten years ago to express the view that the precursor of lactic acid, the so-called lactacidogen, in muscle was of the nature of a hexosephosphate, on the ground of the changes which occurred in the relative amounts of inorganically and organically combined phosphoric acid during the production of lactic acid in contraction. He believed the complex to be a diphosphate of the type isolated by Harden from fermenting mixtures. For some time after this view had been advanced it remained unsupported by any positive evidence of the existence of such a compound, although it received widespread support because of its generally acceptable character. The

last five years have, however, provided much that was lacking, and there can scarcely remain any serious doubts as to the existence and nature of Embden's compound.

Muscle tissue has been shown to possess the same power to synthesise or to hydrolyse hexosediphosphate that we have seen is possessed by yeast juices, and the equations given below illustrate these changes as they probably occur in the contraction and recovery phases in muscle, and make clear the relationship they bear to those for the fermentation of sugar.

Contraction

$$\text{Glycogen} \rightarrow C_6H_{12}O_6$$

$$C_6H_{12}O_6 + 2R_2HPO_4 \rightarrow C_6H_{10}O_4(R_2PO_4)_2 + 2H_2O$$

$$C_6H_{10}O(R_2PO_4)_2 + 2H_2O \rightarrow [C_6H_{12}O_6] + 2R_2HPO_4$$

"Active" sugar

$$[C_6H_{12}O_6] \rightarrow 2CH_3{\cdot}CHOH{\cdot}COOH$$

Oxidative Recovery

$$3CH_3{\cdot}CH{\cdot}OH{\cdot}COOH + 2R_2HPO_4 + 3O_2 = 3CO_2 + 5H_2O + C_6H_{10}O_4(R_2PO_4)_2$$

What, then, is the significance of the intermediate existence of a hexosephosphate in the conversion of sugar to lactic acid in muscle? Are we to believe, from our views on the fermentations of sugars, that the hexose molecule must undergo some type of intramolecular change before it can be degraded by the appropriate agents in the tissues of animals? The parallel naturally attracts us to such an idea, and it is scarcely surprising that the experimental evidence that is rapidly accumulating tends to point in that direction.

All of you will be familiar with the discovery a year or two ago of the internal secretion of the

pancreas by Banting and Best, and with the great extension of our knowledge of the breakdown of sugars in the body which that discovery has brought about. It will be remembered that the administration of the active principle, insulin, to a normal or a diabetic subject leads to a prompt fall in the concentration of reducing sugar in the blood. The rapid improvement in the condition of cases of diabetes which followed the administration of insulin seemed to indicate that the organism had for the time being recovered its power to oxidise glucose, and it was at first thought that the disappearance of sugar from the blood was merely an indication of this utilisation. When carefully controlled experiments were made, however, it was ascertained that the destruction of sugar was not at all comparable with the large amount that had disappeared from the circulating blood, and it was naturally supposed that the excess that could not be accounted for by oxidation had been converted into glycogen or possibly fat. Experiments by Dudley and Marrian, since confirmed by other workers, soon demonstrated that no appreciable proportion of the sugar was converted into these substances under the action of insulin, and for a time the fate of the remaining sugar appeared a mystery.

Last year, however, two Viennese investigators, Audova and Wagner, reported that the injection of insulin resulted in large amounts of sugar being converted in the tissues into a complex resembling the hexosephosphate "lactacidogen." General confirma-

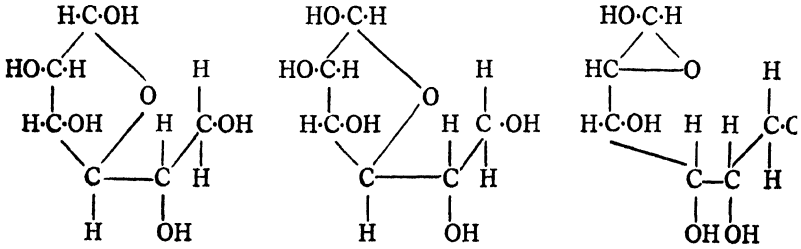
tion of their work was provided by the work of Kay and Robison, who did not, however, believe that the proportion of sugar disappearing from the circulation and accountable for in this manner was quite as large as Audova and Wagner claimed, but who showed that the synthesis occurred largely in the corpuscles at the expense of the inorganic phosphates present there together with additional phosphates drawn from the tissues.

There would seem, therefore, reasonably sufficient grounds for believing that directly or indirectly insulin facilitates the conversion of the blood-sugar into a hexosephosphate, this step being probably a necessary preliminary to its utilisation in the tissues. If we accept this view it naturally occurs to us to enquire whether there is any evidence that by transition through the intermediate complex with phosphoric acid the sugar of the blood is converted into a form that is more readily oxidised, and this at once opens up the closely related question as to the identity of the sugar present in blood.

The Nature of the Sugar in Mammalian Blood

For a considerable number of years it has been generally accepted that the sugar present in the blood and tissue fluids of animals is ordinary glucose, but from time to time occasional doubts as to whether it is all present in this form have been expressed. Usually such doubts lacked any or sufficient experi-

mental support to render them worthy of serious attention, but recently the matter has taken on a different aspect by the researches of Winter and Smith in Hopkins's laboratory at Cambridge. In course of an examination of the bloods of normal and diabetic individuals they made a comparison of the sugar present in the protein-free filtrate as estimated by a copper-reduction method and by polarimetric determinations. It is unnecessary to outline their results in detail here, but it may be said that from the differences which were noted in the two cases they suggested that the sugar of the blood of diabetics is the ordinary α-β-equilibrium mixture that is found in simple, neutral, aqueous solutions. Further, they suggested that the body cells are unable to oxidise this form of glucose with facility, and that it is necessary for the sugar to be converted into a more reactive form, possibly, they thought, the ethylene oxide or γ-form, before it could be broken down.



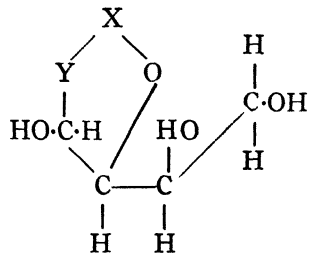
α-Glucose. β-Glucose. γ-Glucose.

The conversion of the relatively inactive α-β-mixture into the more reactive γ-form was, they believed, brought about by the agency of insulin.

These suggestions attracted considerable attention at the time they were advanced, but, unfortunately, their experimental procedure has been rather severely criticised, with the result that their views have not been generally accepted. Nevertheless, evidence is accumulating that their main claim is correct, and that the sugar of the blood is changed before it is oxidised in the organism, and that insulin plays some part in bringing about that change. Recently, Lundsgaard and Holbøll have shown that in the presence of muscle tissue insulin brings about a marked reduction of the rotation of solutions of glucose without affecting its reducing power, a change which they think may be due to the conversion of the α-β-glucose mixture into a variety of that sugar with a specific rotation lower than that of β-glucose.

One gains the impression from their paper that they are not greatly attracted by the suggestion of Winter and Smith that the more reactive form is the ethylene oxide or γ-form of glucose, whilst positive evidence against this view is provided by the interesting researches of Herring, Irvine, and Macleod, who tested a large number of sugars and derived substances with a view to correlating their power to neutralise the effects of the administration of insulin with their molecular structure. Once again we obtain indications of a curious parallel with the fermentation of sugars by yeast that can scarcely be mere coincidence. Of all the substances tested, glucose and mannose are the most powerful restoratives for the condition of insulin hypoglycæmia, whilst fructose is not quite

as potent. From a review of all their results they conclude that there is no exception, which cannot be adequately explained, to the generalisation expressed in the statement that the type of carbohydrate molecule functional in eliminating the convulsion symptoms occasioned by insulin is:



where either X or Y is a reducing group. The three fermentable sugars possessing the common enolic formula (cf. p. 137) conform to this general type.

They were able directly to test the theory of Winter and Smith by showing that glucose monoacetone, a readily hydrolysed derivative of the ethylene form of glucose, is without action when administered in cases of insulin hypoglycæmia.

Phosphates in Relation to Oxidation of Sugars by Hydrogen Peroxide

There are still, it will be observed, many points that are obscure, and it is possible that more direct attack of the problem by experiments *in vitro* will provide a solution. Already this method has yielded a considerable amount of information, but some of it is conflicting and the position is not quite clear.

W. Löb has for some time past claimed that phosphates exert a specific accelerative effect on the oxidation of sugars, particularly glucose, in simple solution by hydrogen peroxide, and he has been strongly supported by the experiments of Witzemann, who believes that the effect is due to the intermediate formation of a hexosephosphate. On the other hand, Harden and Henley believe that the effect is due solely to the phosphates acting as buffers in regulating the neutrality of the medium. In view of this disagreement further work would appear to be necessary, particularly in the light of the observations of Meyerhof and Weber, who find that glucose is not appreciably oxidised at the surface of Warburg's active charcoal model (cf. p. 98), but that hexosephosphate is broken down. Curiously, these investigators found that fructose is unaffected at the charcoal surface, an observation that tends to support the idea that, although fructose can be isolated from the hexosephosphate isolated from fermentation mixtures, it does not represent the hypothetical " active " sugar naturally liberated from the ester.

In the field of plant chemistry progress has been very much slower, but already a certain amount of evidence is being collected together which tends to show that phosphoric acid is intimately concerned in many of the changes which carbohydrates undergo in the germinating seed or the developing plant. In this connection the observation of Ling and Nanji that one of the two major constituents of the

starch grain is present as a phosphoric ester is of considerable significance.

Phosphates in Relation to Calcification of Cartilage

Leaving this aspect of the rôle of phosphoric acid in the cell after what must be regarded as a very incomplete review, I wish to pass on to consider with you an interesting development of Harden's work on the hexosephosphates of fermentation mixtures. Together with Robison, he succeeded in isolating from such material a hexosemonophosphate which is quite distinct from the monophosphoric ester obtained by Neuberg by partial hydrolysis of the ordinary diphosphate. It was found to yield soluble salts with barium and calcium, and in course of studying the action of enzymes on these salts Robison observed that they were hydrolysed with the deposition of calcium or barium phosphate. The thought at once occurred to him that such compounds might be related to the deposition of lime in bone formation. Experiments soon showed that cartilage actually possesses the power to hydrolyse this monophosphoric ester and the development of his early observations has proved to be of extraordinary interest.

The first task was to demonstrate that compounds of this type are present in the circulating blood, and Goodwin and Robison were able to do this last year. Two esters were detected, but not with

sufficient certainty to enable their structure to be recognised. Of these, one shows reducing properties, is optically lævorotatory, and is rapidly hydrolysed by an enzyme found in ossifying cartilage, the kidney, and the gut, whilst the other does not reduce and is unaffected by what may be termed the bone enzyme.

Let us examine how these discoveries have revolutionised our views on the calcification of cartilage, and have at last given us a reasonable conception of the mechanism of this process that is acceptable by the physical chemist as well as by the biochemist. The difficulty of explaining the process by which calcium is deposited as phosphate and carbonate in developing bone and not in other tissues has in the past led to a considerable amount of wild speculation. Some of the views have, from the fact that they were based on a certain amount of experimental work, attracted some attention, but it was difficult to arouse much enthusiasm over suggestions such as that of Pfaundler, that the process is one of specific adsorption of ions, or that of Freudenberg and György, according to which calcification takes place in the following stages:

Cartilage protein + calcium = Cartilage protein-calcium
Cartilage protein-calcium + phosphate = Cartilage protein-Ca-phosphate
Cartilage-protein-Ca-phosphate = Calcium phosphate + cartilage protein

It is refreshing to turn to Robison's clear analysis of the condition under which calcification probably occurs.

In the first place, brief reference must be made to

what is known concerning the condition of calcium in the blood. It will be recalled that Cushny found some years ago that serum filtered through collodion showed unequal passage of ions, and that, generally speaking, the inorganic constituents pass through, with the exception of a certain proportion of calcium and magnesium, which is apparently present in a colloidal or semi-colloidal form. Further work has supported his observations, so that it is now generally recognised that both these elements are present to a considerable extent in serum in a form which will not pass a dialysing membrane with ease. It is not known in what form this material is present, but it is suspected that it is of the nature of a complex with protein. Even if we disregard for the moment the calcium which is present in this form and consider that which is believed to be present in simple solution, we are faced with the fact that more is present than could be dissolved in the same volume of water, in other words, that it may be necessary to regard serum as a supersaturated solution as regards calcium. It is in this light that some attempts have been made to explain calcification, but as Robison and Soames point out, it is not justifiable to do this until much more is known of the factors influencing the degree of ionisation and the solubility product. A supersaturated solution might, it is true, deposit calcium phosphate on coming into contact with the solid phase, i.e. bone, but this would not explain the beginning of ossification in embryonic cartilage. If, however, for the sake of argument we regard

the serum and body fluids as saturated solutions, there are definite conditions which *must* lead to a deposition of calcium phosphate and carbonate. One of these is a rise in the concentration of phosphate or carbonate ions, and this will occur if there is an increase in the concentration of inorganic phosphates or a change of reaction towards the alkaline side.

The first of these conditions is set up if hydrolysis of a soluble hexosephosphate takes place by the agency of the bone enzyme, a process which, as we have seen, undoubtedly takes place.

The possibility of a change in the reaction of the tissue fluids occurring at centres of ossification must also be considered, because Robison has ascertained that the bone enzyme shows optimum activity between pH 8·4–9·4; a reaction very much more alkaline than that associated with ordinary tissues. It is impossible yet to say whether *in vivo* the osteoblasts possess the power to raise the alkalinity in their immediate neighbourhood, and so enable the enzyme to work in the most efficient manner, but a few, and admittedly insufficient, experiments indicate that they do.

Bassett has already shown that the solid phase in equilibrium with solutions containing phosphate and calcium under conditions found in normal serum is the compound hydroxyapatite, $[Ca_3(PO_4)_2]_3 \cdot Ca(OH)_2$. This compound is more basic than tertiary calcium phosphate, a fact that weakens the suggestion of Howland and Kramer that the solubility product,

$CaHPO_4$, is the limiting factor in the deposition of bone.

Robison's work, developing as it has from the study of an apparently unimportant product isolated from the fermentation products of sugar, provides another striking example of the far-reaching importance which such discoveries may have if only the observer has the requisite breadth of vision.

I would like to extend my discussion of the condition of calcium and phosphoric acid in the blood, not only to indicate how Robison's work must enlarge our knowledge of the absorption and excretion of calcium and phosphates, but also to a treatment of the extraordinarily interesting and important results which have recently been obtained in the study of rickets and tetany. Unfortunately, time will not permit, and even if opportunity were available, I fear I might be led far from the main subject of this lecture.

On second thoughts, however, I am not so sure that we would stray so far afield. Reading the modern work on the relation between rickets and certain forms of infantile tetany, bearing in mind the curious changes which are noted in the calcium and phosphorus of the blood in these conditions, pondering over Collip's isolation of the internal secretion of the parathyroid glands and the effect this substance has in raising the calcium of the blood, in some cases to so high a level that the animal dies from the resulting hypercalcæmia, and then recalling the hypoglycæmia so often associated with

the parathyroid tetany, one gains the impression that before long these conditions will be found to be very closely related. It would be unwise to do more than to predict this development, but I believe it will take place, and that the metabolism of carbohydrates is intimately bound up with phosphoric acid and calcium, and that the conditions of rickets, osteomalacia, parathyroid tetany, "milk-fever" in cattle, and disturbances of sugar breakdown are more intimately related than we think at present.

LECTURE IV

THE VITAMINS

It will be understood from what was said in the last lecture that the satisfactory deposition of lime-salts in developing bone must be dependent on factors which control both the amount of calcium and phosphorus in the blood, and the form in which these elements are present. One of these factors is now known to be an organic substance of undetermined nature which is found in certain natural foodstuffs, and which is somewhat generally referred to as the antirachitic vitamin. In the absence of this substance there is imperfect calcification of cartilage in the young animal, usually associated with a marked fall in the amount of calcium and phosphorus in the blood, even when the diet may contain ample amounts of these elements for the normal requirements of the species.

How this substance exerts its action is at present unknown, and, indeed, it must be admitted that we know practically nothing about the mode of action in the body of any one of the four or five food constituents that to-day are called vitamins.

I would first of all direct your attention to a brief consideration of what we mean by a vitamin. The classic researches of Hopkins demonstrated for the

first time that higher animals are unable to grow normally and show satisfactory development and health unless their diet contains certain components of natural foods that apparently do not belong to the four main classes of food substances, the proteins, fats, carbohydrates, and inorganic salts. A striking feature of his experiments was the demonstration that the newly discovered substances are effective in amounts extraordinarily small as compared with those of other foodstuffs. Funk coined for these substances the name vitamine, which, with the dropping of the letter *e*, so as not to imply that they are nitrogenous substances of a basic character, has been widely adopted in the form *vitamin*.

Following on Hopkins's pioneer work an immense mass of literature has accumulated in this field of research, and not only have five separate substances essential for the growth and well-being of the higher animals been satisfactorily differentiated one from the other, but a large number of other less clearly defined substances have been described as being essential for the growth of various types of living creatures. A review of this mass of work, much of which is unfortunately open to severe criticism, gives us an opportunity to draw up some sort of a definition of what we mean by a vitamin.

In the present state of our knowledge we may reasonably regard a substance as a vitamin if it is organic in nature, does not belong to the proteins, fats, or carbohydrates, and is essential in relatively

small amounts for the nutrition of an organism which does not possess the power to synthesise it.

Having thus attempted to define a vitamin, although frankly one cannot feel very proud of the definition that has been given, and which has been given only because one is so often asked what the name stands for, it might be well if we pass on to consider how many of the substances that have been so described satisfy these requirements. It will be convenient if we review very briefly only the more outstanding work, and begin with that which has been carried out with the lower organisms.

The first work which attracts our notice is that reported by Allen and Nelson in 1910–1914, when they stated that minute traces of a substance present in sea-water and apparently organic in character are necessary for the growth in pure culture of certain marine diatoms. Almost at the same time a series of papers appeared from the laboratory of the late Prof. Bottomley on the presence of substances, termed by him *auximones*, in peat which had been allowed to undergo changes induced by inoculating with cultures of certain soil organisms. These substances were, he claimed, essential in extremely small amounts for the growth of a number of green plants.

If organisms so simple as the diatoms of Allen's experiments, or the duckweed used by Bottomley, and possessing a fully developed chlorophyll system enabling them to synthesise their own organic matter from inorganic materials, are dependent upon traces

of pre-formed organic matter, then one is left wondering whether in the progress of evolution even green plants have lost their autotrophic nature, and whether any organisms have survived which can live in a purely inorganic environment. To my mind it appears unlikely that simple green plants can have lost this power, and a careful re-examination of the work that has just been referred to indicates that this view is probably correct. Miss Peach, working in my laboratory, has been able to show that the marine diatom *Nitzschia closterium* can be grown without any difficulty in synthetic media prepared from pure inorganic salts, provided care be taken to regulate the balance of the various constituents, whilst Clarke, in America, has in a similar manner shown that Bottomley's failure to obtain growth of *Lemna* in inorganic media was entirely due to faults in the composition of the nutritive fluids he employed.

Whilst it is true that these researches seem to have disproved the necessity of vitamins or "*auximones*" for green plants there remain certain indications that small traces of organic substances may exert a stimulating influence on their growth. It is difficult to give an opinion on this matter in the present state of our knowledge, for it has not yet been ruled out that the effects of the additions to the culture fluids may not be due to traces of certain elements not formerly present. In this connection the work carried out at Rothamsted on the stimulating action of traces of boron on the growth of broad beans, as well as the researches of Mazé on the effect of traces

of other unusual elements on the growth of green plants, is of great interest.

It is soon apparent from a survey of the confused literature in this field that investigators have failed to differentiate between substances essential for life and growth and those which exert a mere stimulant action. This is particularly well apparent when one reads the many papers that have been published upon the growth of yeast in pure cultures. As far back as 1901 Wildiers demonstrated that growing cultures of yeast contain a substance, to which he gave the name "bios," which in very small amounts was necessary for the growth of yeast in a synthetic medium when the implantation was a small one. A large inoculation usually grew without any separate addition of "bios" because, Wildiers thought, it carried with it a sufficient supply of that factor. This work virtually reopened the famous controversy begun between Pasteur and Liebig, and carried on by Mayer and v. Nageli, and has led to a very large amount of research being undertaken.

Time and time again as one reads the papers that have resulted from this work one realises how often confusion between a substance essential for growth and one causing a stimulation of growth has arisen. Undoubtedly, substances are produced during the growth of yeast which, when added to new cultures, tend to stimulate cell multiplication, but the careful experiments of Fulmer, Nelson and White seem to make it clear that *Saccharomyces cerevisiæ* can grow in a synthetic artificial medium prepared from

inorganic salts and pure sugars, even when for the latter one uses the synthetic sugar, methose. This result may indicate either that yeast growing in such a medium can itself produce the substance which stimulates its own growth, or that the growth of the organism does not require such a substance. In the former case the stimulating substance cannot be regarded as a vitamin.

One outcome of this work has been that a number of investigators, having shown that yeast synthesises the substance known as vitamin B when growing in pure cultures, have been led to identify this substance with the hypothetical "bios." So far, however, the most reliable work is definitely against this view, and it would appear that the two substances are quite distinct.

Regarding the bacteria the facts are less clearly defined than in the case of the yeasts. Certain species such as the sulphur bacteria, studied with such care by Waksman and his colleagues, and the nitrite bacteria, appear able to flourish in a wholly inorganic medium, and to be quite independent of pre-formed organic matter. This is in agreement with what is known concerning their strictly autotrophic character. The vast majority of bacteria, lacking this power to synthesise organic materials from carbon dioxide by utilising the energy made available by simple exothermic inorganic oxidations, are dependent to a greater or a lesser extent on organic foodstuffs. For many species it has been claimed that in addition they require minute amounts

of certain growth stimulants akin to the vitamins (Lloyd, Shearer), particularly in the case of the so-called hæmophilic bacteria (Legroux and Mesuard). A close examination of this work, however, immediately impresses one with the unsatisfactory nature of much of the experimental evidence presented, and one feels that too often the easy alternative of suggesting the action of a "vitamin" has been accepted in place of facing the difficult task of determining more accurately the nutritive requirements of the particular organism.

A particularly interesting example of the necessity of attempting to make clear the nature of these requirements before concluding that vitamins are necessary for bacterial growth has just been encountered in our own laboratory, where it has been found by Miss Reader that the extraordinarily powerful action which as little as one part of tryptic broth in 4,000 parts of an artificial medium containing very pure inorganic salts and glucose exerts on the growth of certain bacteria is a complex one, due, apparently, in part to the addition of certain inorganic ions, in part to supplying certain substances which can act as hydrogen acceptors, and, probably, in part to what, for lack of a better term, we call a "bios" effect. Certainly the substance, seemingly organic in nature, which produces the last-mentioned effect is not essential for growth, but exerts a stimulating action comparable with the effect of Wildiers' "bios" on yeast, or that of Bottomley's *auximones* on green plants.

When we turn to the animal kingdom, having passed by cases of symbiosis between plant and plant and between plant and animal, where the conditions are obviously too complex to investigate in the present state of our knowledge, we find the information available rather less confusing and more reliable. Investigations of the nutritive requirements of the simplest animals seem to show that certain protozoa, at any rate, can be grown for many generations in artificial culture media free from any substances resembling the vitamins. Peters has maintained the ciliate organism *Colpidium colpoda* for over a year in pure culture in a medium in which the only organic material was ammonium glycero-phosphate.

T. B. Robertson insists, however, that during the growth of the protozoon *Enchelys* a substance is found which stimulates cell-division of this organism. Indeed, on the basis of this work he has built up a most elaborate theory of the autocatalytic nature of cell growth (*The Chemical Basis of Growth and Senescence*, London, 1923).

Attractive as much of his speculation appears at first sight, it must be remembered that, as yet, the experimental foundation is insecure. Cutler and Crump could obtain no indication of his autocatalytic —or, as he prefers to call it, allelocatalytic—effect when studying the growth of *Colpidium*, but, on the other hand, they appear to find that the addition of small quantities of substances similar in nature to "bios" have a marked effect on the growth of this organism.

The theory of allelocatalysis has also been rejected by Peskett in a series of carefully conducted experiments on yeasts carried out in Peters's laboratory at Oxford. The only difficulty regarding the researches on the growth of protozoa, and it is one to which Robertson persistently fails to give sufficient attention, is that their normal food is bacteria, which seem to form substances of the " bios " type as do the yeasts.

Once again we must attempt to distinguish between the essential and the non-essential factors, although when one essays to do this the difficulties are at once apparent. If we do not exclude substances such as " bios," which are believed to be produced by the cell itself, we shall have to discuss not only the essential vitamins, but a host of other factors which in one sense or another might be regarded as important for the organism ; such, for example, as the minute trace of malic acid, which will cause the larva of the wood-boring organism *Toredo* to swim towards the wood which is to be the home of the mature animal (Harrington), or the infinitesimally small traces of substances of unknown character which Carrel has shown are produced by leucocytes growing in pure culture, and which appear necessary for the growth of fibroblasts.

Returning to our studies of the vitamins we find the next animal, higher in the evolutionary scale, to have been investigated is, as far as I am aware, the fruit fly, *Drosophila.* Here we have clear evidence derived from the carefully planned experiments of

Loeb and Northrop, as well as Harden and Bacot, that the substance known as vitamin B is essential to the development and growth of the larvæ. Apparently the other vitamins required by higher animals are not essential for this species.

Studies on the tadpole and frog have shown that whereas vitamin B is absolutely essential, there may be some dependence on vitamin A, but probably none on the antiscorbutic substance C (Emmett and Allen, Harden and Zilva). Fish require both A and B; the birds show dependence on both A and B factors, but the C substance is of doubtful importance, whilst for the mammals not only are the former substances essential, and in some cases the antiscorbutic factor also, but there appears to be a dependence on other as yet less clearly defined factors, such as the antirachitic vitamin, and the substance necessary for reproduction discovered by the careful work of Evans and Bishop.

These few facts tend to support the view that in the progress of evolution the animal becomes more and more dependent on certain definite molecular structures synthesised by plant agency, and one recalls the words of Hopkins, spoken when, at a meeting of the Society of Public Analysts in London in 1906, he first announced the discovery of the substances we now term vitamins: "The animal body is adjusted to live either on plant tissues or other animals, and these contain countless substances other than proteins, carbohydrates, and fats. Physiological evolution, I believe, has made some of these

wellnigh as essential as are the basal constituents of the diet."

In presenting this brief and somewhat incomplete review of the dependence of living organisms on the vitamins, I have attempted to present an aspect of this subject which too frequently is lost sight of, in the hope that it will stimulate much-needed work to resolve the factors which influence cell growth, particularly of the lower animals and plants, for we can scarcely hope to gain a knowledge of the development of the higher animals whilst we are almost wholly ignorant of those governing the growth of the unicellular organisms.

Having dealt with only one of the many physiological aspects of the vitamins I wish now to turn to discuss for a few moments what is known of their chemical nature, for we are at last beginning to gain some reliable information on this most important subject.

Vitamin A and the Antirachitic Vitamin D[1]

I propose to deal with these two substances together since we have recently obtained some evidence that they may be related chemically.

If one takes any natural substance containing one

[1] The term vitamin D has been applied by Funk and Dubin (*loc. cit.*) to the substance which stimulates the growth of yeast. Since this substance appears to be not essential it is not well classified as a vitamin. The letter D should be applied to the antirachitic factor which McCollum and his co-workers have satisfactorily differentiated from the growth-promoting vitamin A.

or both of these factors and subjects it to hydrolysis by means of a boiling alcoholic solution of caustic alkali under conditions preventing oxidative changes, one can extract both vitamins unchanged as regards physiological activity by light petroleum or ether.

The unsaponifiable matter prepared from cod-liver oil, the usual raw material employed on account of its richness in both substances, represents a little under 1 per cent of the original oil, but contains all the vitamin activity. Oxidation of this material rapidly destroys the growth-promoting substances A and rather more slowly the antirachitic factor D. By crystallisation from methyl alcohol at low temperature and by subsequent precipitation with digitonin all the cholesterol present may be removed without affecting the vitamin activity of the residue, which is a red-brown oil corresponding to about 0·4 per cent of the original cod-liver oil. Attempts to isolate active fractions from this material are of little use, owing to the presence of considerable amounts of resinous substances formed during saponification. These may be removed by subjecting the material to distillation in superheated steam in an atmosphere of nitrogen. This purified material appears on analysis to consist very largely of unsaturated alcohols, probably allied to the hydro-aromatic or terpene group. On fractional distillation in high vacuum a number of fractions may be obtained, of which the most interesting are:

(*a*) B.p. 180–200°/1 mm. Consists chiefly of an unsaturated alcohol, together with a small amount

of a highly unsaturated hydrocarbon similar to spinacene. This fraction has as yet been examined very little, as it contains only traces of growth-promoting or antirachitic activity.

(*b*) B.p. 200–240°/2 mm.

(*c*) A fraction boiling between 260° and 300° at this pressure.

The growth-promoting substance A is definitely associated with fraction (*b*), and, from some preliminary experiments which have been recently carried out, it appears to contain the antirachitic factor as well.

This fraction is obviously impure and contains at least three recognisable substances in addition to the vitamins. These substances are: (i) A certain amount of the unsaturated alcohol which constitutes the main fraction of (*a*); (ii) a considerable amount of a highly unsaturated hydrocarbon closely resembling the spinacene or squalene, previously reported by Tsujimoto and by Chapman as a constituent of certain fish-liver oils; (iii) a small amount of a saturated alcohol which can be separated in the pure condition as white, pearly, laminated crystals, m.p. 58–60°, and which is probably identical with the batyl alcohol of the Japanese investigators.

Fraction (*c*) consists mainly of the solid alcohol together with considerable amounts of the hydrocarbon.

During the past year we have been attempting to obtain evidence that will tell us whether any one of these substances can be identified with either vitamin A or D, but so far we have only been able to show

that the solid alcohol and spinacene have no apparent physiological action, and that it is unlikely that the unsaturated alcohol in fraction (*a*) or the highly unsaturated hydrocarbon resembling spinacene will replace either vitamin in the diet. We are, therefore, inclined to believe that the vitamins, particularly vitamin A, are present in fraction (*b*) in relatively small amount.

Even if this fraction were one of the vitamins in a pure condition a simple calculation makes clear how exceedingly small is the amount required by an animal in comparison with its ordinary foodstuffs. A rat of 100 g. eats daily about 3 g. of protein, 2 g. of fat, and 10 g. of carbohydrate. To supply the vitamins A and D it is only necessary to give the rat about 5 mg. of cod-liver oil daily. This corresponds to approximately 0·05 mg. of unsaponifiable matter, and to 0·005 mg. of the active fraction (*b*). If the vitamins are, as we suspect, merely impurities present in that fraction the dose becomes vanishingly small. These vitamins will withstand acetylation and benzoylation, but apparently not saturation of the ethylene bond of the alcohol; oxidative changes destroy their activity.

If we are correct, as I fear we are, in believing that the vitamin A constitutes only a small proportion of the active fraction of our distillate, it certainly renders our chances of isolating it very much more slender, for the chemical manipulation of such material presents considerable difficulties.

At any rate, however hard our task, we have the

satisfaction of knowing that we are dealing with clearly defined chemical units and not elusive substances such as the enzymes. The active fractions contain only carbon, hydrogen, and oxygen. The crystalline material prepared by Takahashi, and claimed by him to be the active substance, has been shown by us to be a crude mixture of the type I have described. His view that the vitamin is an aldehyde is based on inadequate evidence, and although it would fit in with the ready destruction by oxidation, is not supported by the resistance of the active substances to vigorous hydrolysis with alkalies.

It might also be mentioned that the curious property which certain oils have of causing the reduction of phosphomolybdic acid can be transferred to the unsaponifiable fraction with, however, a distinct loss of the power. Dr. Rosenheim and I have satisfied ourselves that this reaction, whether it is due to aldehydes or other reducing substances, certainly does not run parallel with the vitamin A activity. We have, however, also satisfied ourselves that the long-known colour reaction given by cod-liver oils on treatment with strong sulphuric acid is in all probability due to the active principle A. We have ascertained that the blue-purple colour produced by this reagent is also produced by a variety of other substances, in particular by arsenic chloride, dimethyl sulphate, or trichloroacetic acid, and that the intensity of the coloration is not only an indication of the amount of vitamin A present, but that with these last-mentioned reagents the colour lasts sufficiently

long to enable one to make an approximate determination of its intensity by matching against a series of standards prepared from dyestuffs. Such estimations, inaccurate as they are, are probably more trustworthy, and certainly very much more convenient than the tedious animal-feeding tests.

Vitamin D

Our efforts to isolate from the unsaponifiable matter of cod-liver oil the principle which plays so important a part in the formation of bone were interrupted by the discovery that a much more convenient source of the active substance is available.

It will be recalled that for some time past there has been a conflict of opinion regarding the etiology of rickets. On the one hand, there have been those who regarded the disease as the direct result of defective hygienic surroundings, particularly lack of sunshine and fresh air, whilst, on the other, there was the school of thought that believed a faulty diet to be the main causative factor.

For some time there appeared to be no obvious path by which a reconciliation between these divergent views would be effected, but a discovery by Huldschinsky that exposure of a rachitic child to ultra-violet light or direct sunshine would result in a prompt cure has, by its subsequent development, shown us how this aim can be attained.

Much of the progress in this field is due to Steenbock at Wisconsin Experimental Station, and it was he that showed that exposure of a mixture of food

substances, which, untreated, would induce rickets in animals, to the radiation of a mercury-vapour lamp endowed the ration with antirachitic properties. Obviously one of two alternative explanations of this remarkable fact are available. Either the exposure of the skin of a child or of the rickets-producing diet to ultra-violet radiations brings about a synthesis of the active substance, or, after the exposure, a secondary radiation is emitted by the treated material which is responsible for the antirachitic effect. At first there were indications that the second alternative was the correct one, but the experiments on which this view was founded have been shown to be faulty ; the supposed secondary radiations being actually the emission of a phosphorescence from the silica vessels used in the test.

On the other hand, there is now evidence that the former of the two explanations describes what actually happens. Dr. Rosenheim and I were able to show simultaneously with Steenbock, and also Hess in America, that the active substance is produced under the action of ultra-violet light from cholesterol.[1] Pure cholesterol (m.p. 148·7°), prepared with great care and crystallised many times from different solvents, becomes powerfully antirachitic after a few hours' exposure to ultra-violet light. The change does not appear to be an oxidative one,

[1] Dr. Rosenheim and I at first thought that radiation of cholesterol by ultra-violet light had formed the growth-promoting vitamin. Later work by Rosenheim and Webster demonstrated, however, that it is an antirachitic substance that is formed.

because it occurs equally well in an inert atmosphere or a high vacuum. It is, we think, a chemical change, for Dr. Rosenheim has succeeded in removing the majority of the unchanged cholesterol by means of precipitation with digitonin, and in recovering a minute fraction of material not precipitated by that reagent but very powerfully antirachitic. The chemical investigation of this fraction will be awaited with great interest and optimism, for it is obvious that the chances of isolating and identifying the active substance are very much better when one starts from a clean, pure material such as cholesterol, than when one is obliged to use a complex mixture such as the unsaponifiable matter from cod-liver oil.

It is interesting to speculate that both vitamin A and the antirachitic vitamin, of which latter substance it may be said that it no longer comes under the definition we have given of a vitamin, since it is synthesised by the animal organism under the action of short-length light waves, are probably related to cholesterol.

Vitamin E

This substance is the dietary factor described a year or two ago by Evans and Bishop, and it is convenient to discuss it here because it appears to be of the same type of substance as the two we have just described. An inadequate supply of this factor in the diet leads to a failure of reproduction in rats, and the work of the investigators we have mentioned clearly differentiates its action from that of the other

vitamins. As yet no full details are available as to its nature or properties, but it occurs in a number of oils and fats (e.g. wheat oil), and it may be recovered in the material resisting saponification with alkalies. As far as I am aware no further information as to its chemical character has yet been published.

Vitamin B

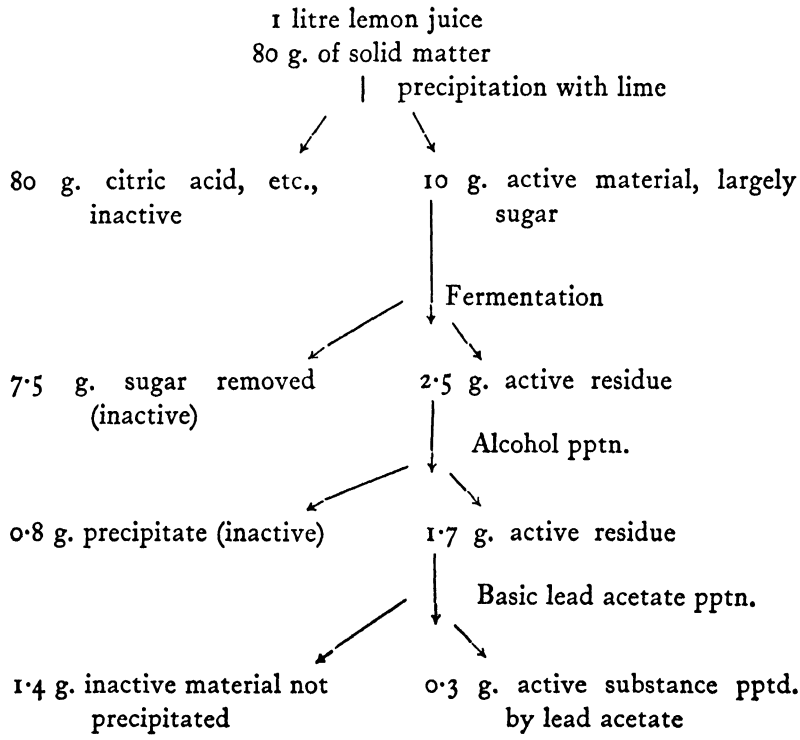
No one of the vitamins has been more studied from the chemical side than this substance. In spite of this we are still in almost complete ignorance of its chemical nature. The most recent claim of isolation is that put forward by Bertrand and Seidell, who describe the separation of the active substance in the form of a picrate, of which a daily dose of some milligrams will protect pigeons from beri-beri. The picrate can be separated by crystallisation from water into two picrates, melting at 202° and 160° respectively, the latter being the more active physiologically. Dr. Seidell informs me that he believes the active substance to be a pyrimidine. This will recall the similar belief held by Funk and by Edie, Evans, Moore, Simpson and Webster, concerning the substances they isolated. Confirmation of the results of Bertrand and Seidell is urgently required.

Vitamin C

Curiously, this factor, although apparently of relatively simple character, has until recently received practically little or no attention from chemists.

During the last year or two, however, the painstaking researches of Dr. Zilva at the Lister Institute have given us a good deal of valuable information regarding its properties.

He has shown that it is readily destroyed by oxidation when in solution reacting on the alkaline side of neutrality, and that its molecule is probably not much larger than that of a hexose. His attempt to isolate the active principles may be illustrated by the scheme outlined below.



The last stage he has reached, namely the basic lead acetate fractions, contains extremely small

amounts of nitrogen and phosphorus, but possesses approximately the whole of the vitamin activity. It represents 0·03 per cent of the original juice.

If more time were at my disposal I would direct your attention to other phases of present-day vitamin research, in particular to the important studies that are being made to ascertain the rôle of these interesting substances in the life of the organisms. One would also like to devote a short time to passing in review the extraordinary developments which have followed the recent application of knowledge regarding the vitamins to the practical problems of human nutrition and animal husbandry.

One cannot help feeling, however, that the most urgent problems are those concerning the chemical nature of the vitamins, for only when reasonably pure preparations of the active substances are available can we expect to gain a clear knowledge of their physiological action in the body.

Blood and Circulation from the Standpoint of Physical Chemistry

By Laurence J. Henderson

TITLE OF LECTURES

LECTURES

I. The Physico-chemical Changes in Blood during the Respiratory Cycle

II. The Synthetic Description of Blood as a Physico-chemical System

III. Deductions concerning the Circulation

LECTURE I

THE PHYSICO-CHEMICAL CHANGES IN BLOOD DURING THE RESPIRATORY CYCLE

IT is the task of the physiologist not merely to analyse the phenomena of organic activity and to define the several physico-chemical processes which underlie them, but also to discover how these processes interact with one another and how, as a result of such interactions, the harmonious unity of the organism is assured. In this problem of the integration of the physico-chemical activities of the living being we may discern one of the primary differences between the biological and the physical sciences. Yet this difference is often overlooked. For, on the one hand, we are all disposed through absorption in our narrow researches to disregard the bearing of discoveries in other fields upon our own problems. And, on the other hand, it is all too common for the devotees of the experimental method, which is necessary but insufficient in physiology, to fail to understand that the interpretation of physiological observations sometimes necessitates the use of a method different from that which suffices in physical science. Such one-sidedness is, however, hardly excusable since the publication of the works of Claude Bernard.

The great French physiologist was not merely

one of the earlier apostles of the experimental method in the biological sciences. Fortified by his laboratory experience, he also undertook a philosophical study of physiology and clearly defined several of the most general and fundamental characteristics of the phenomena of life. As a result of his labours the field of physiological research was greatly enlarged.

Among the wider views developed by Claude Bernard two seem to me especially important: First, his opinions concerning the existence of a peculiar and unfailing harmonious unity in vital phenomena, not less among those of a physico-chemical character than among those which we associate with the older physiology. Secondly, his hypothesis concerning the existence of a constant internal environment in the higher animals. To these two conceptions he returned again and again in his writings, but I think that two quotations, freely translated, will suffice to explain his mature thought.

"Admitting that vital phenomena rest upon physico-chemical activities, which is the truth, the essence of the problem is not thereby cleared up; for it is no chance encounter of physico-chemical phenomena which constructs each being according to a pre-existing plan, and produces the admirable subordination and the harmonious concert of organic activity.

"There is an arrangement in the living being, a kind of regulated activity, which must never be

neglected, because it is in truth the most striking characteristic of living beings. . . .

" Vital phenomena possess indeed their rigorously determined physico-chemical conditions, but, at the same time, they subordinate themselves, and succeed one another in a pattern and according to a law which pre-exist ; they repeat themselves with order, regularity, constancy, and they harmonise in such manner as to bring about the organisation and growth of the individual, animal, or plant.

" It is as if there existed a pre-established design of each organism and of each organ such that, though considered separately, each physiological process is dependent upon the general forces of nature, yet taken in relation with the other physiological processes, it reveals a special bond, and seems directed by some invisible guide in the path which it follows and toward the position which it occupies.

" The simplest reflection reveals a primary quality, a *quid proprium* of the living being, in this pre-established organic harmony."

And again :

" For the animal there are really two environments ; an external environment in which the organism is placed, and an internal environment in which the cells live. Life goes on, not in the external environment, air, fresh water, or salt water, as the case may be, but in the liquid internal environment composed of the organic circulating liquid which surrounds and bathes every cell. This medium is composed of the lymph and the plasma,

the liquid portion of the blood, which in the higher animals penetrate the tissues and make up the totality of the interstitial liquids. These are the instrument of all the local nutritive processes, the source and confluence of the exchanges of the cells. A complex organism must be considered as a union of simple beings, its cells, which live in the liquid internal environment.

"The constancy of the internal environment is the condition of free and independent life. The mechanism which makes possible this constancy assures in the internal environment the maintenance of all the conditions necessary to the life of the cells. Thus we may understand that there can be no free and independent life for those simple beings whose [active] cells are in direct contact with the cosmic environment, but that this form of life is, on the contrary, the exclusive privilege of such beings as have reached the height of organic complexity and differentiation.

"The constancy of the environment presupposes such a perfection of the organism that at every moment external variations are compensated and equilibrated."

It is permissible, on the one hand, to regard both these ideas of Claude Bernard's as fundamental, or, on the other hand, to consider the constancy of the internal environment as only a peculiarly important and interesting example of the more general phenomenon of organisation. There is, however, a practical advantage in continually insisting upon

the more concrete idea, the constancy of the internal environment, not less than upon the more abstract idea, organisation. To-day it seems desirable, with the help of modern discoveries, to seek a more complete explanation of the constancy of the internal environment, a phenomenon which in some respects was divined rather than clearly understood by Claude Bernard. But this is impossible without a description of many instances of harmonious and integrated organic activity.

At the outset we are confronted by a fact which may seem to contradict the hypothesis of constancy itself. There are, indeed, two kinds of blood, arterial and venous, in the body. They are different even to the naked eye. But this difficulty is more apparent than real, for, as we shall see, there take place in the blood certain processes which are more important than the others in their physico-chemical consequences. We shall accordingly find, in studying the nature of physico-chemical equilibria of the blood, that these equilibria permit a remarkable autonomous regulation of certain important physico-chemical properties and also ensure the large variations in the content of oxygen and carbonic acid which are essential for active metabolism. Thus we may form the opinion that constancy of hydrogen-ion concentration, and of osmotic pressure or water concentration, are of peculiar importance, and, again, that within limits chloride may be substituted for bicarbonate in the plasma, if certain secondary adaptations are assured, without injury to the cells.

The hypothesis of constancy of the internal environment does not, however, at all imply identity of the aqueous solutions bathing the cells in all parts of the body. Rather it is a question of a steady state characterised by variations from place to place, but also by constant gradients between different places. Nor does it, of course, imply absolute constancy. It is possible to conceive only an approximation to constancy of composition and of gradient. On the whole, however, the closeness of this approximation is highly remarkable, and the tendency to restore equilibria which have been disturbed is one of the most striking characteristics of the organism.

Our subject may now be defined. We shall study, first, the several physico-chemical phenomena which are known to be involved in the respiratory cycle of the blood; next, the manner in which these phenomena are inter-related and integrated to constitute a process which is one of the important physiological functions; then, at length in possession of the more important quantitative data, we shall re-examine the question of the constancy of the internal environment for the normal individual in a steady state, and finally we shall seek a higher physiological synthesis of the physico-chemical processes in blood with the activities of heart, lungs, and capillaries. This synthesis must still remain very incomplete.

* * * * *

Our knowledge of the physico-chemical equilibria in blood which are involved in the respiratory process

rests upon experimental studies of the following subjects:

(1) The heterogeneous equilibrium between gaseous oxygen and blood.
(2) The heterogeneous equilibrium between gaseous carbon dioxide and blood.
(3) The acid-base equilibrium in plasma.
(4) The distribution of bicarbonate between cells and plasma.
(5) The distribution of chloride between cells and plasma.
(6) The distribution of water between cells and plasma.

I

Oxygen is absorbed by blood almost exclusively as a consequence of a chemical reaction between hæmoglobin and oxygen such that one atom of iron in the hæmoglobin molecule corresponds to two atoms of oxygen. This reaction, as you well know, has given rise to much discussion. For a pure dilute solution of hæmoglobin the phenomenon corresponds approximately, but, I think, not exactly, to the requirements of the equation

$$k = \frac{[Hb]}{[HbO_2]}[O_2]$$

which expresses the conditions for equilibrium of the chemical reaction

$$HbO_2 = Hb + O_2$$

Nevertheless, the true nature of this reaction

remains somewhat uncertain. It is in any case exceptional. Thus oxygen is a simple substance of molecular weight thirty-two, while the lowest possible molecular weight of hæmoglobin is more than five-hundredfold greater, a happy *mésalliance*, but one which may yet give rise at least to a vague uncertainty. And, in fact, the size of the hæmoglobin molecule, the formation of colloidal aggregates in the presence of salts, the influence of the ionisation of its carboxyl and amino-groups upon the affinity for oxygen, and other related problems remain unsettled. However this may be, it is certain that the equilibrium between oxygen and blood is markedly different from that between oxygen and pure hæmoglobin solutions. In blood the conditions are represented approximately by Hill's well-known equation, which may be expressed in conventional physico-chemical form as follows:

$$k = \frac{[\mathrm{Hb}]}{[\mathrm{HbO_2}]}[\mathrm{O_2}]^n$$

Here the value of n, always much larger than one, is a characteristic of each specimen of blood.

The real physiological phenomenon is, however, not a simple physico-chemical process, and we may well, for the present purpose, confine ourselves to the facts. It is possible to express these very simply by two curves, which sum up the results of Ferry (3) and of Bock, Field and Adair (4).

The absorption of oxygen by blood may be regarded as the result of a well-defined evolutionary

adaptation which makes possible the transport of oxygen in large amounts. The combination of carbonic acid with the constituents of blood, while no less highly fitted to the needs of the organism,

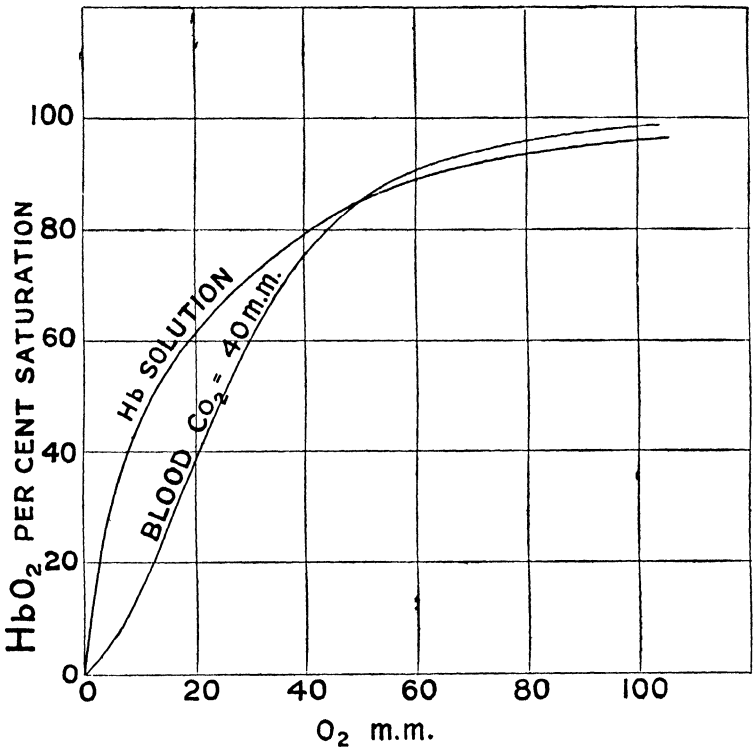


FIG. 1.—OXYGEN ABSORPTION CURVES.

rests upon a more primitive chemical reaction. This reaction is inevitable, as a result of the general characteristics of the metabolic process. It occurs not only in blood, but also in all other parts of the body, and, indeed, generally, throughout animals and plants. In blood there is only one peculiar

phenomenon involved—the influence of hæmoglobin upon the carbonic acid equilibrium, to which we shall return.

Oxygen combines with a single substance which is present only in the corpuscles. Carbonic acid, like acids in general, combines with all the bases of plasma and of cells. Thus there is no specific agent for the fixation of carbonic acid, and this is a function of the alkalinity of the blood. If we represent the salts of other weak acids in blood by BA, the fixation of carbonic acid may be said to depend upon a disturbance of the equilibrium of the reaction

$$BA + H_2CO_3 = BHCO_3 + HA$$

The acid radicals in question are almost exclusively those of the proteins. Furthermore, the quantity of carbonic acid contained in arterial blood is never greatly inferior to that in venous blood, whereas there may be, in certain cases, very large differences in the case of oxygen. Again, the capacity of blood to combine with oxygen is strictly limited by its hæmoglobin content, and arterial blood is, in fact, nearly saturated in this respect. But the content of carbonic acid in blood is capable of almost indefinite increase as the pressure of carbon dioxide increases, there being no sharp upper limit to the capacity to combine with carbonic acid. The concept of blood saturated with carbonic acid is, accordingly, meaningless.

Finally, carbonic acid, free and combined, possesses certain other important functions, aside from its

respiratory function. For example, it plays a peculiarly important rôle in the preservation of the constancy of the internal environment. Oxygen, on the other hand, which is present in the plasma in very small amounts, in solution in the free state, is only indirectly involved in such phenomena.

It is also important to note that on account of greater solubility the concentration of free carbonic acid in blood is far greater than that of free oxygen. The concentration of combined carbonic acid is also greater than the concentration of combined oxygen.

In spite of these differences, the absorption curves of the two substances are not altogether dissimilar. That of carbonic acid, however, shows no tendency to become horizontal within normal ranges of pressure, and has no trace of S-shape.[1]

The roughly similar forms of the two curves correspond to the physiological functions of the two substances. In the case of oxygen it is the upper, more horizontal portion of the curve which is important in the lung. It is easy to see that even with wide variations of oxygen pressure there may be very nearly complete saturation of blood with oxygen. The lower portion of this curve, which approaches the vertical, represents conditions which may exist in the tissues. Here, until the supply of oxygen is almost exhausted, the head of oxygen

[1] For the sake of consistency all the data to be considered in these lectures are taken from the papers of Bock, Field and Adair, and of Henderson, Bock, Field and Stoddard, *J. Biol. Chem.*, 59, pp. 353–431, 1924.

must remain appreciable, since it falls very slowly for a large decrease in combined oxygen.

In the case of carbonic acid the difference between arterial and venous bloods is less marked. But it is clear that, even if imperceptibly, the same differences in inverse order are present.

All these differences between the two cases may

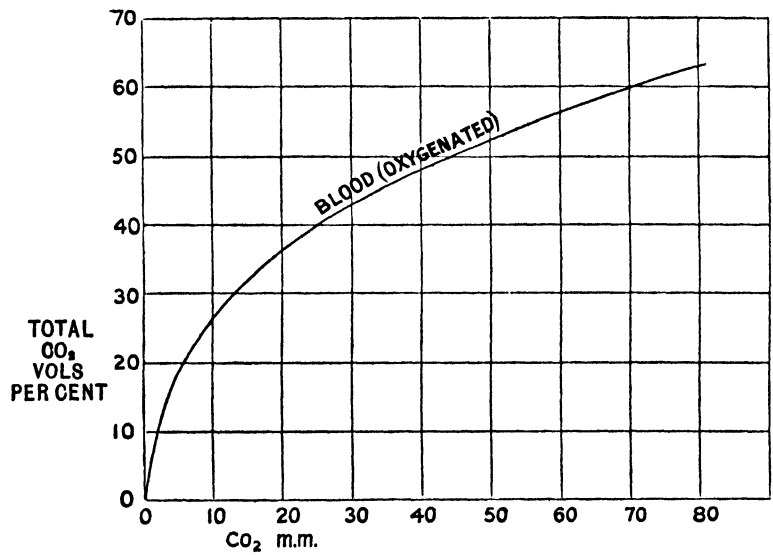


FIG. 2.—A CARBON DIOXIDE ABSORPTION CURVE.

be summed up by the representation upon the same figure and on the same scale of the two familiar dissociation curves.

On this figure the physiological ranges are enclosed by two rectangles.

It is easy to see on Fig. 3 that the difference in absorbed carbonic acid corresponding to the ordinary difference in carbon dioxide pressure between arterial

and venous blood is only about one-half the corresponding difference in the case of oxygen. Such

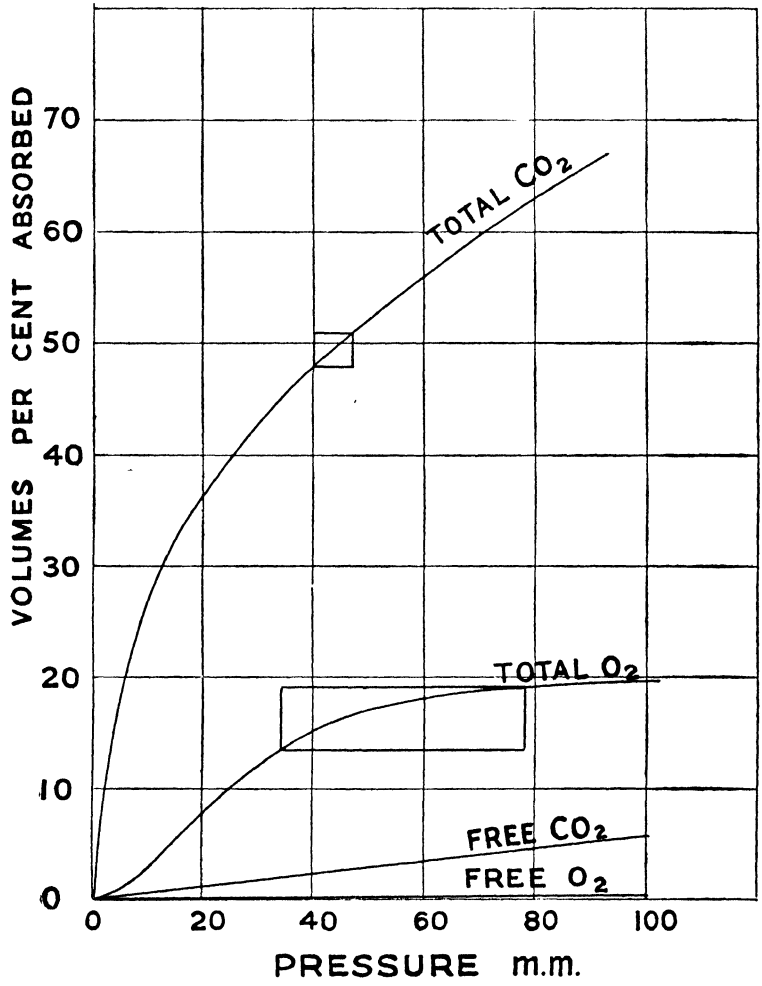


FIG. 3.—OXYGEN AND CARBON DIOXIDE ABSORPTION CURVES.

a state of affairs, implying a respiratory quotient of about 0·5, is manifestly impossible. This paradox, which has been more or less apparent for many

years, might long ago have pointed the way to progress. But it was overlooked.

II

From the standpoint of the physiologist the facts thus far considered are but half-truths, because, other important complications aside, there is in the blood an interaction between oxygen and carbonic acid. Long ago Bohr, Hasselbalch and Krogh proved that increase in concentration of free carbonic acid is accompanied by dissociation of oxyhæmoglobin. Later the subject was carefully studied by Barcroft, whose important investigations have settled the problems involved. The phenomena revealed by Barcroft's investigations may be illustrated by a diagram showing the oxygen dissociation curves of blood at several different pressures of carbonic acid.

The curves of this figure, reading from left to right, correspond to pressures of carbon dioxide of 3, 20, 40, and 80 mm. respectively for the blood of A.V.B. (4). These curves are all approximately similar, and each is not far from agreeing with Hill's equation:

$$k = \frac{[Hb]}{[HbO_2]}[O_2]^n$$

Here k is obviously a function of the pressure of carbon dioxide, and it may readily be shown that for Barcroft's original results the value of k is approximately expressed by the following equation:

$$k = \frac{[H_2CO_3] + 7{\cdot}7}{0{\cdot}014}$$

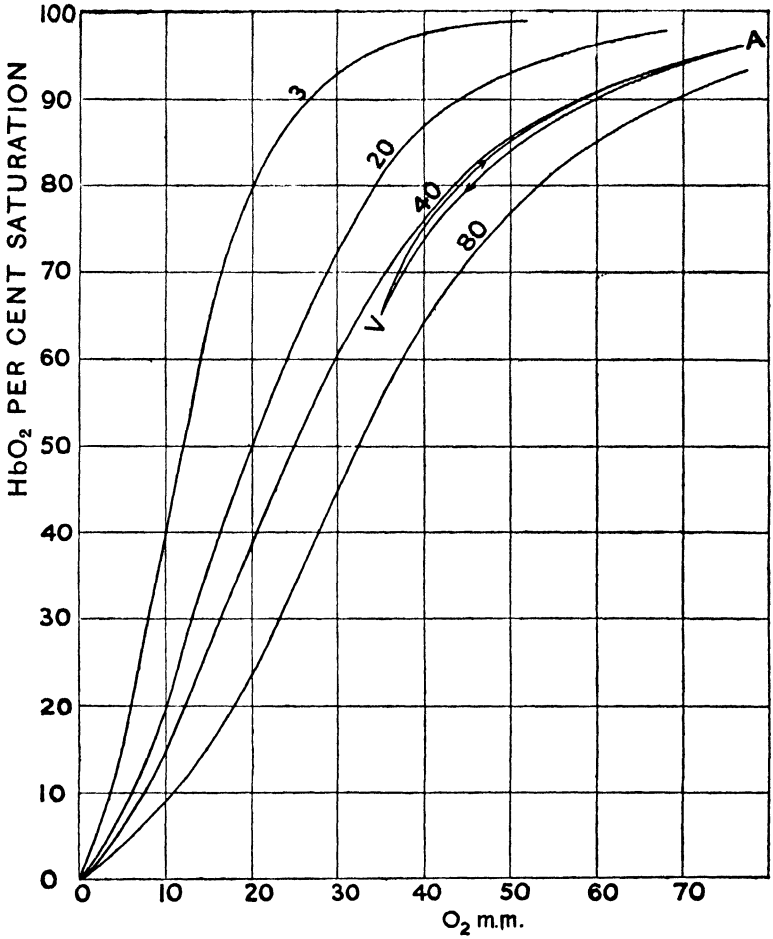


FIG. 4.—OXYGEN ABSORPTION CURVES OF BLOOD AT 3, 20, 40 AND 80 MM. CO_2 PRESSURE.

Therefore the facts concerning the absorption of oxygen may be represented by some such equation as the following:

$$\frac{H_2CO_3 + 7{\cdot}7}{0{\cdot}014} = \frac{[Hb]}{[HbO_2]}[O_2]^{2{\cdot}5}$$

The results of Bock, Field and Adair correspond

to a similar, if slightly more elaborate, algebraic expression, but the difficulty need not be considered here ; this is a question of detail.

It is very important at this point to insist upon two simple conclusions from the facts. First, the capacity for oxygen of a given sample of blood at a given pressure of oxygen is only constant when the pressure of carbon dioxide is also constant. This capacity varies in a perfectly definite manner with the pressure of carbon dioxide. Secondly, the fall in concentration of carbonic acid in the lung is accompanied by a rise in the capacity of the blood to absorb oxygen, and the rise in concentration of carbonic acid in the tissues is accompanied by a fall in this capacity.

Therefore, the physiological process of oxygen transport is not represented by one of this family of oxygen dissociation curves but by a different curve or cycle which cuts across them, as shown in Fig. 4.

In the present state of knowledge it seems strange that the physiologists who interested themselves in these questions should not have drawn from these researches the conclusion that, since carbonic acid influences the oxygen equilibrium in blood, oxygen must influence the carbonic acid equilibrium. A mere glance at the equation which sums up Barcroft's results is sufficient to prove the point, if, indeed, proof of so obvious a conclusion is necessary. Yet such is the inertia of the mind that this conclusion escaped us all, and it remained for Christiansen, Douglas and Haldane to prove experimentally that

the carbon dioxide absorption curves of oxygenated and of reduced bloods are different.

The curves corresponding to various degrees of oxygenation are now known to fall at levels corresponding to the relative amounts of oxyhæmoglobin which may be present.

On account of the less direct theoretical interest

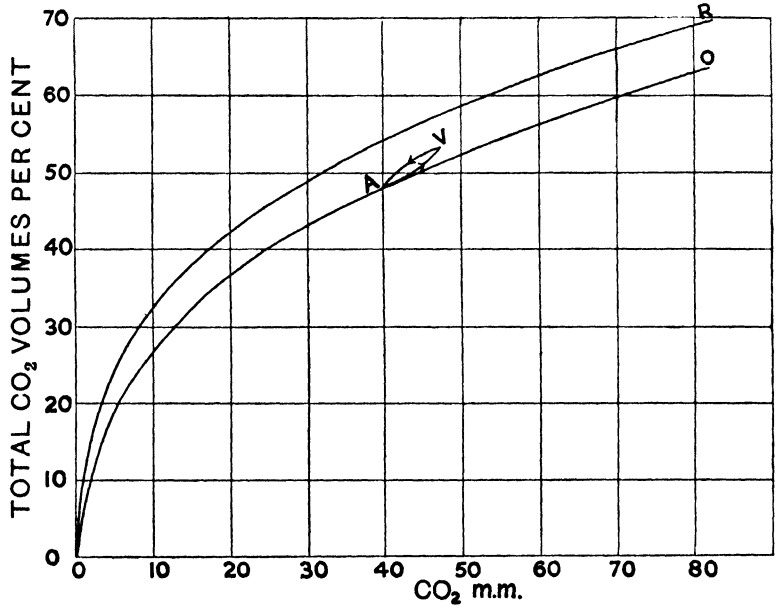


FIG. 5.—CARBON DIOXIDE ABSORPTION CURVES OF REDUCED (R) AND OXYGENATED (O) BLOOD.

in these curves it has not become the custom to seek an analytical expression to represent them. The relationship between the logarithms of the concentrations of free and combined carbonic acid is, however, almost precisely linear.

Evidently the physiological process in the case of carbonic acid, as in the case of oxygen, is not

represented by one of these curves, but by another curve, or rather cycle, which cuts across them. The normal cycle is shown on Fig. 5.

In studying the interaction between carbonic acid and oxygen in the blood it is of great importance not to think of the action of one substance as cause and of the changes in the other as effect. This error, one of the most familiar of fallacies, and one of the most natural, was undoubtedly responsible for the long delay in reaching a correct understanding of the phenomenon. There is, in fact, a perfectly reciprocal relationship between the two substances. Thus in the lung the escape of carbonic acid favours the absorption of oxygen, and the latter process favours the elimination of carbonic acid. In the tissues the inverse relationship obtains. There is, however, a considerable difference between the magnitudes of the two interactions when expressed in terms of oxygen and carbonic acid absorbed or eliminated. Under ordinary circumstances the exchanges of carbonic acid are more favoured than are those of oxygen by the interaction, e.g. in the ratio of nearly ten to one for the lung. We shall return to this question.

In order to understand the mechanism of this phenomenon it is now necessary to extend the scope of our discussion. The problems thus far considered have been investigated more particularly by the British and Danish Schools of Physiology, while the solution of the problems of acid-base equilibrium in blood is in large part the result of American researches.

A weak acid, HA, dissociates incompletely in aqueous solution to form H and A ions:

$$HA = \overset{+}{H} + \overset{-}{A}$$

For this reaction the law of mass action yields the equation:

$$k = [\overset{+}{H}] \frac{[\overset{-}{A}]}{[HA]}$$

If a salt of the weak acid be also present, the following equation is approximately true:

$$k' = [\overset{+}{H}] \frac{[\text{salt}]}{[\text{acid}]}$$

$$\text{or } [\overset{+}{H}] = k' \frac{[\text{acid}]}{[\text{salt}]}$$

In words, the true reaction of the solution is proportional to the ratio of free acid to salt in solution. The value of k', the constant of the last equation, is slightly greater than that of k, the ionisation constant of the acid in question.

For carbonic acid in blood plasma the value of k' is $7{\cdot}6 \times 10^{-7}$, the hydrogen-ion concentration is about $0{\cdot}37 \times 10^{-7}$N., and the ratio of free to combined carbonic acid is approximately 1 : 20. In short, the reaction of blood plasma is very faintly alkaline, the concentration of hydroxyl ions being roughly ten times that of hydrogen ions.

Carbonic acid is the chief factor in the regulation of the reaction of blood and tissues. It owes its importance in this respect to the precise degree of its strength as an acid. As a result of this property,

the magnitude of which may be most conveniently measured by the ionisation constant, not only the aqueous solutions of the organism, but also sea-water and natural waters generally, are of very constant and nearly neutral reaction. The stabilisation of reaction is, however, greatly increased through the universal presence of carbonic acid in the atmosphere, and on account of the approximately equal distribution of this substance between gas and water phases. These conditions depend upon the magnitude of the solubility of carbon dioxide in water.

The regulation of the reaction of blood is, then, even more primitive than the processes involved in the chemical absorption of carbonic acid by blood, and although the actual conditions of the blood which control the reaction are highly adapted to physiological needs, the phenomenon is in origin by no means an evolutionary adaptation, but, on the contrary, an unchanging characteristic of our terrestrial environment, solely dependent upon the properties of water and carbon dioxide. This fact was the starting-point of studies of the fitness of the environment which I long ago undertook and which have convinced me that so-called adaptations are often not exclusively evolutionary products.

In the case of blood the mechanism by which carbonic acid operates to preserve constant the hydrogen-ion concentration may be roughly explained without difficulty. It should be noted, in the first place, that the concentration of free carbonic acid is not commonly liable to great fluctuations

because the activity of the lung tends to prevent these; and, secondly, that since the concentration of bicarbonates in blood is very high, relatively large changes are unlikely except when great quantities of acid are involved. Let us now imagine the addition to blood of a quantity of hydrochloric acid equivalent to one-half its total bicarbonate, an enormous quantity. Then the reduction of the quantity of combined carbonic acid would be less than one-half, there would be momentarily a large increase of free carbonic acid, but, after the excess of free acid had been excreted by the lung, the ratio of free acid to salt would become about 1 : 10, and the hydrogen-ion concentration $0{\cdot}74 \times 10^{-7}$N. But, since the respiratory centre is peculiarly sensitive to even very slight changes of reaction, the concentration of free carbonic acid must continue to fall, and may approach very close to one-half its original value. At this point the hydrogen-ion concentration itself will have regained its normal magnitude. We may represent this process schematically in the following manner:

	Free Carbonic Acid.	Bicarbonate.	$\frac{[\text{Acid}]}{[\text{Salt}]}$	$[\overset{+}{\text{H}}]$
First stage .	1	20	0·05	$0{\cdot}37 \times 10^{-7}$N.
Second stage	11	10	1·10	$8{\cdot}14 \times 10^{-7}$N.
Third stage	1	10	0·10	$0{\cdot}74 \times 10^{-7}$N.
Fourth stage	0·5	10	0·05	$0{\cdot}37 \times 10^{-7}$N.

The principles of the acid-base equilibrium may be applied to the phenomenon discovered by

Christiansen, Douglas and Haldane. Given the data necessary for the construction of a carbonic acid dissociation curve, it is possible to calculate approximately the hydrogen-ion concentration of

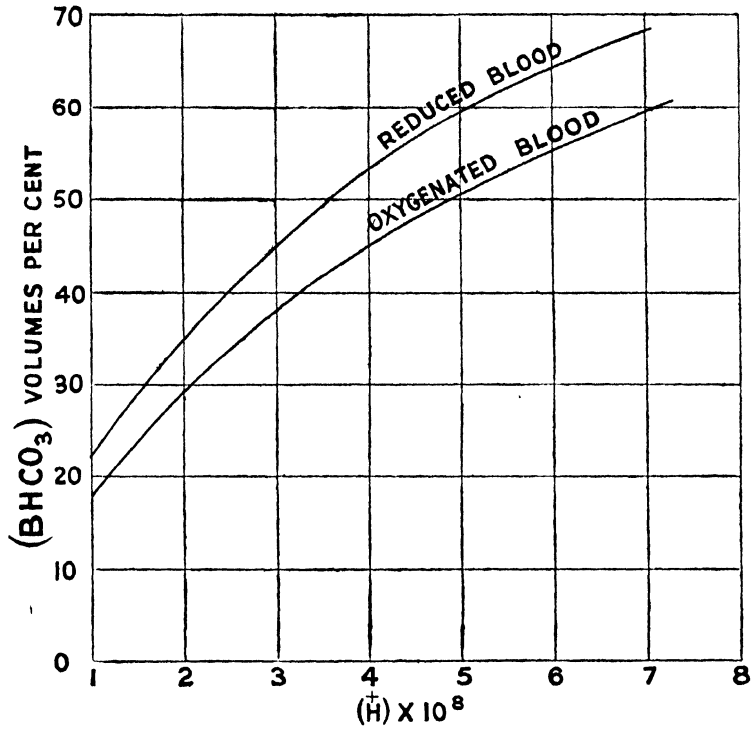


FIG. 6.—COMBINED CARBONIC ACID AS A FUNCTION OF HYDROGEN-ION CONCENTRATION IN REDUCED AND OXYGENATED BLOOD.

the blood. Without additional information, precision is, indeed, impossible, because of the complications of the heterogeneous equilibrium between corpuscles and plasma, but this is a difficulty that may be postponed. Thus it is possible to express the combined carbonic acid in blood as a function,

not of the carbon dioxide pressure, but of the hydrogen-ion concentration.

Fig. 6 expresses the results of this calculation. The curves closely resemble those from which they have been derived, but they permit an important deduction. Evidently the points of the two curves which fall on the same abscissa correspond to the same hydrogen-ion concentration. Now the curve for reduced blood is everywhere above that for oxygenated blood. It follows that, hydrogen-ion concentrations being equal, reduced blood always combines with more carbonic acid than oxygenated blood. How is this possible? The only chemical reaction of oxygen in blood is the formation of oxyhæmoglobin. Therefore oxyhæmoglobin must be either a more acid or a less alkaline substance than reduced hæmoglobin. The mechanism of this process is still uncertain, but the fact is beyond doubt. If we assume that in the hæmoglobin molecule an acid radical becomes more acid upon oxygenation, as a result of the presence of oxygen in the molecule, it is possible to explain the interaction of oxygen and carbonic acid in blood. Under these circumstances oxygenation, by increasing the acidity of hæmoglobin, must be followed by a decomposition of bicarbonate. But, if combination with oxygen influences the acid radical in question, a change in the ionisation of this radical must, in turn, exert an action upon the affinity of hæmoglobin for oxygen.

In the tissues, on the other hand, when the con-

centration of carbonic acid increases and all the weak acid radicals give up acid to this substance, the affinity for oxygen must diminish and oxygen must be set free. Meanwhile increased acidity of hæmoglobin tends to make compensation for the loss of carbonic acid, diminished acidity of hæmoglobin, in turn, for increased concentration of carbonic acid, thus further stabilising the alkalinity of the blood.

Once more it is important to take note that a reciprocal action is involved. In the lungs the hæmoglobin molecule undergoes a change which is favourable alike to the absorption of oxygen and to the elimination of carbon dioxide. In the tissues the reverse process takes place. We should insist upon the idea of action and reaction, not of cause and effect.

A crude but useful representation of the process is given by the accompanying diagram.

There are still other phenomena involved in the respiratory cycle of the blood, some of which present new and interesting problems. Years ago it was observed by Zunz that variation in the pressure of carbon dioxide is accompanied by change in the distribution of electrolytes between corpuscles and plasma. It is now known that the movement of two substances is chiefly responsible for this phenomenon; one of these is water, the other is the chloride ion. Moreover, it was easy to foresee, after the results above expounded had become known, and it has been proved by McLean (5), that the

exchange may be induced by variations of oxygen pressure.

This phenomenon is due to changes of osmotic pressure, i.e. of water concentration, and to changes of alkalinity, which follow upon changes in carbonic acid or oxygen concentrations. It is evident that such changes must be different in cells and in plasma

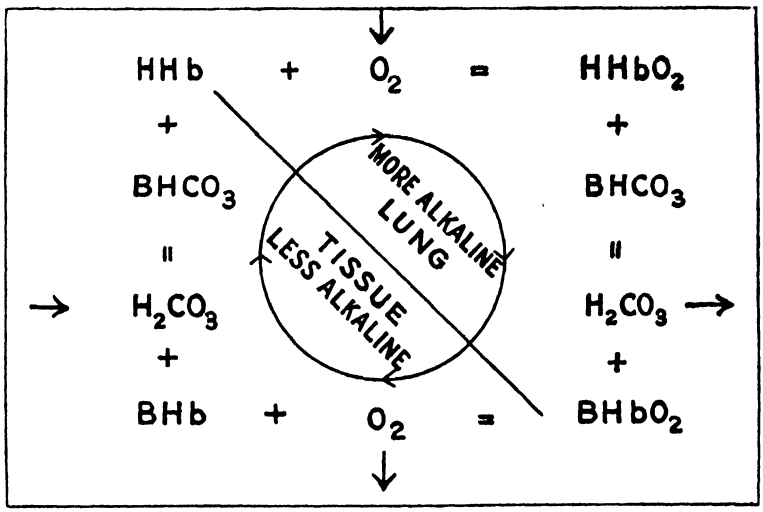


FIG. 7.—CHEMICAL REACTIONS OF THE RESPIRATORY CYCLE.

owing to differences in the composition of the two phases. As a result the pre-existing heterogeneous equilibrium must be disturbed and exchanges must follow tending towards a new condition of equilibrium.

As long ago explained, this phenomenon is nothing but a complex example of heterogeneous acid-base equilibrium (6). It is, however, complicated by certain factors which have recently come to light. One of these is the peculiar behaviour of hæmoglobin.

Another is the fact that the exchanges involve, at least in sensible quantity, only water and anions.

It has been shown by Van Slyke, Wu and McLean (7) that the state of heterogeneous equilibrium between red cells and plasma agrees, at least approximately, with the requirements of Donnan's theory. In other words, the following equations are approximately true:

$$\frac{[\bar{Cl}]_c}{[\bar{Cl}]_s} = \frac{[\bar{HCO_3}]_c}{[\bar{HCO_3}]_s} = \frac{[\bar{OH}]_c}{[\bar{OH}]_s} = \frac{[\overset{+}{H}]_s}{[\overset{+}{H}]_c} = r$$

Here r is the Donnan coefficient, and the subscripts c and s refer to corpuscles and serum (or plasma) respectively.

Such are the physico-chemical phenomena which occur in blood during the respiratory cycle. Presented even in this summary and elementary form they seem almost bewildering in their complexity, but still more in their interactions. Each of these phenomena involves familiar problems of physical chemistry, but the problem of their interactions, though no less a physico-chemical problem than the others, is also, and in a manner quite different, a problem of physiology, that is to say, of integration. Accordingly, when these interactions have been properly described we shall find that the physico-chemical activities of the blood accompanying respiration well illustrate the two principles of Claude Bernard with which we began this lecture. All the changes are but parts of a single physico-chemical process which we shall now study.

LECTURE II

THE SYNTHETIC DESCRIPTION OF BLOOD AS A PHYSICO-CHEMICAL SYSTEM

THE conception which we have reached of the respiratory activity of the blood is dissatisfying because incomplete. It is also inadequate to our needs, as the frequent failure of those who have established the facts to understand their full significance attests. Let us try to lay bare the essence of the difficulty.

Our knowledge of the phenomena involves relations between seven variables: $[\overset{+}{H}]$, $[H_2CO_3]$, $[BHCO_3]$, $[O_2]$, $[HbO_2]$, $[H_2O]$ or, more conveniently, v (the volume of the corpuscles), and r (the Donnan ratio). All facts apply to samples of blood the total composition of which is liable to no change except such as may be caused by changes in oxygen and carbon dioxide pressures.

This knowledge may be summed up as follows: First, the equilibrium involving the variables $[\overset{+}{H}]$, $[H_2CO_3]$, and $[BHCO_3]$ in plasma or corpuscles may be expressed by the equation $[\overset{+}{H}] = k' \frac{[H_2CO_3]}{[BHCO_3]}$. This law, established long ago, has been confirmed by the exact researches of Hasselbalch and of Van Slyke. Beyond question, it holds with great accuracy for the plasma.

Secondly, the equilibrium involving the variables $[O_2]$, $[HbO_2]$, and $[H_2CO_3]$ may be expressed by a contour line chart.[1] This fact was fully established by the researches of Barcroft. But this chart has as its analytical expression an algebraical equation between three variables. According to the work of Barcroft, the enlarged Hill's equation already cited (replacing [Hb] by the expression $1 - [HbO_2]$ and inserting the value of n) :

$$\frac{[H_2CO_3] + 7{\cdot}7}{0{\cdot}014} = \frac{1 - [HbO_2]}{[HbO_2]}[O_2]^{2{\cdot}8}$$

is the correct expression of the facts.

Other researches lead to slightly different results, but this is a matter of detail.

Thirdly, the equilibrium involving the variables $[H_2CO_3]$, $[BHCO_3]$, and $[HbO_2]$ may be represented by a contour line chart [2] which corresponds to an equation between these three variables. This is the result of the research of Christiansen, Douglas and Haldane.

Finally the investigations of Henderson and McLean and of Van Slyke, Wu and McLean prove that the relationships between the variables $[\overset{+}{H}]$, $[HbO_2]$, and r and between $[\overset{+}{H},]$ $[HbO_2]$, and v may be represented in a similar manner.

Thus it is evident that the facts which are known concerning the blood equilibria may be expressed, in every case, by an equation of the form :

$$f(x, y, z,) = 0$$

[1] Fig. 4 above. [2] See Fig. 5 above.

More specifically, all the facts which have been experimentally established for the respiratory equilibria in blood may be expressed by the following equations:

$$f_1([\overset{+}{H}], [H_2CO_3], [BHCO_3]) = 0 \quad . \quad . \quad . \quad . \quad (1)$$
$$f_2([O_2], [HbO_2], [H_2CO_3]) = 0 \quad . \quad . \quad . \quad . \quad (2)$$
$$f_3([H_2CO_3], [BHCO_3], [HbO_2]) = 0 \quad . \quad . \quad . \quad . \quad (3)$$
$$f_4([\overset{+}{H}], [HbO_2], r) = 0 \quad . \quad . \quad . \quad . \quad (4)$$
$$f_5([H], [HbO_2], v) = 0 \quad . \quad . \quad . \quad . \quad (5)$$

Stated in this form the problem becomes clearer. Evidently each of these equations, when stated explicitly, e.g.:

$$[H] = k' \frac{[H_2CO_3]}{[BHCO_3]} \quad . \quad . \quad . \quad . \quad . \quad (I)$$

is a partial description of the equilibrium, but, since some of the variables of each equation recur in at least one other equation, all five equations are interdependent. Therefore one of them, taken by itself, can but mislead us concerning the totality of the phenomenon.

Thus, when many years ago Barcroft established the diagram which defines the relations between oxygen, hæmoglobin, and free carbonic acid in blood, and I the equation of the acid-base equilibrium, each of us not unnaturally supposed that the problems which we had studied were solved. But this was true only in a very restricted sense, for other variables were involved in the phenomena, and these variables were not, as is so often the case in the highly abstract researches of the physicists and of the chemists, of

secondary importance. On the contrary, they were no less important than the subjects of our investigations. In fact, we were studying different aspects of the same problem, but we did not know it. The essential nature of the phenomenon escaped us.

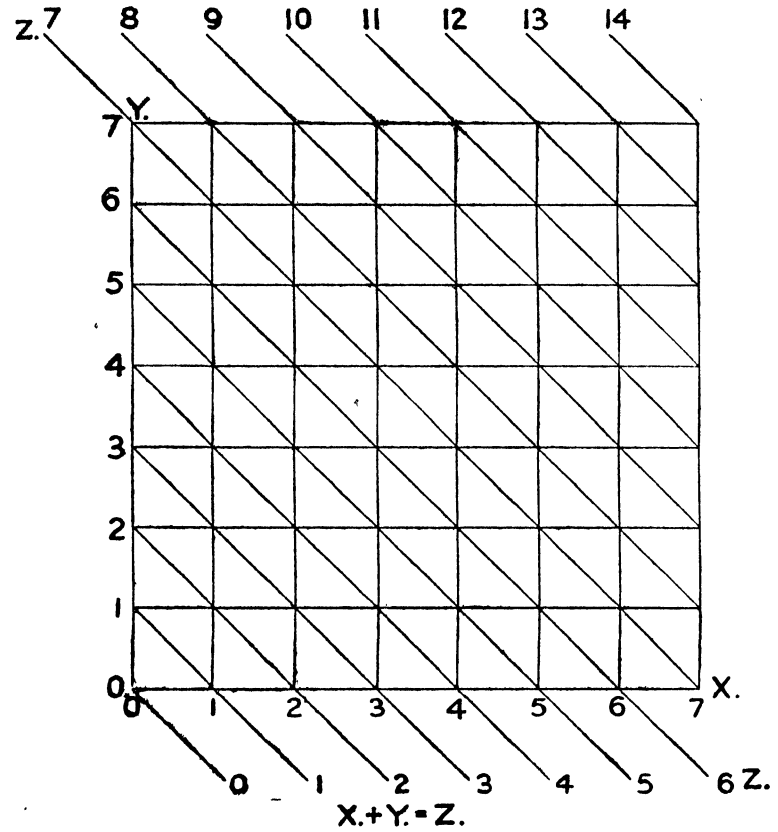


FIG. 8.

The five equations above written state, moreover, only a small part of what may be deduced from them. There are, in fact, thirty-five combinations among seven objects, taking three at a time.

Therefore, there are, in all, thirty-five possible analogous expressions concerning the respiratory changes in blood. It is a matter of chance that five in particular were first discovered.

The nature of the case may be illustrated with the

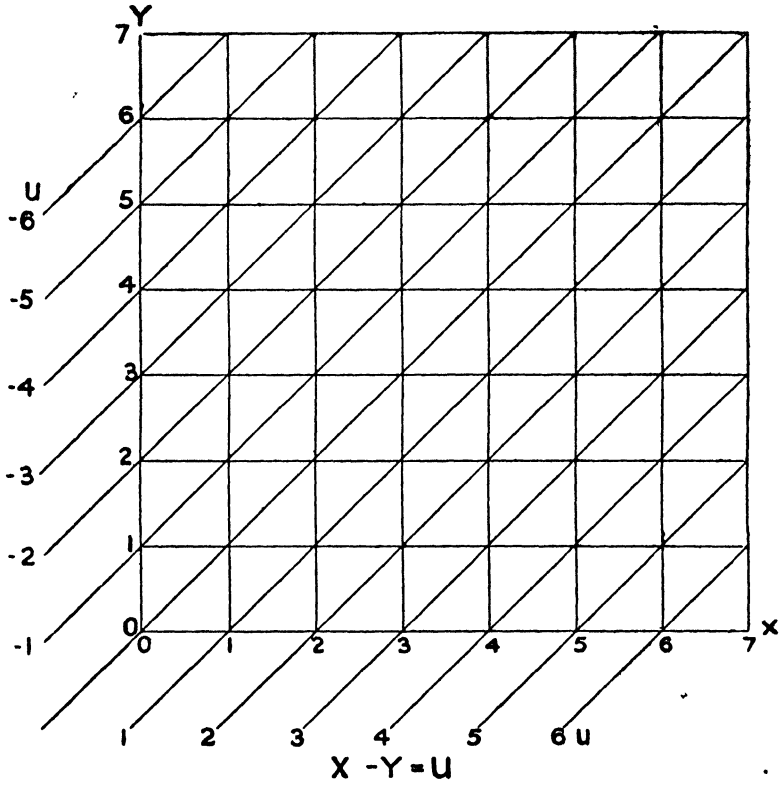


FIG. 9.

help of an example taken from elementary mathematics. Consider the five equations:

$$x + y = z$$
$$x - y = u$$
$$x + 2y = v$$
$$x - 2y = w$$
$$x^2 + y^2 = r^2$$

Each of these equations corresponds to a contour line chart which may be readily constructed. For example, Fig. 8 is such a representation of the equation $x + y = z$, and Fig. 9 of the equation $x - y = u$.

It is also easy to obtain from these five equations thirty others, each in three variables. Thus, adding the first two we obtain :

$$z + u = 2x, \text{ etc.}$$

We may next take note that two or more contour line charts, provided they have the same Cartesian co-ordinates, may be superposed, just as one may superpose a geological upon a topographical map, following this, if desired, by a political map.

Fig. 10 is the result of thus combining Figs. 8 and 9. It expresses all that is expressed by these two figures, plus whatever may be deduced from them, when both are known. Thus it is the expression of the four equations :

$$\begin{aligned} x + y &= z \\ x - y &= u \\ z + u &= 2x \\ z - u &= 2y \end{aligned}$$

It would be a simple matter to continue this process, first constructing contour line charts from the three remaining equations :

$$\begin{aligned} x + 2y &= v \\ x - 2y &= w \\ x^2 + y^2 &= r^2 \end{aligned}$$

and then superposing these upon Fig. 10. The resulting nomogram, the representation of which

presents no particular interest, must evidently define simultaneously all that the thirty-five possible contour line charts or the thirty-five equations in three variables can yield.

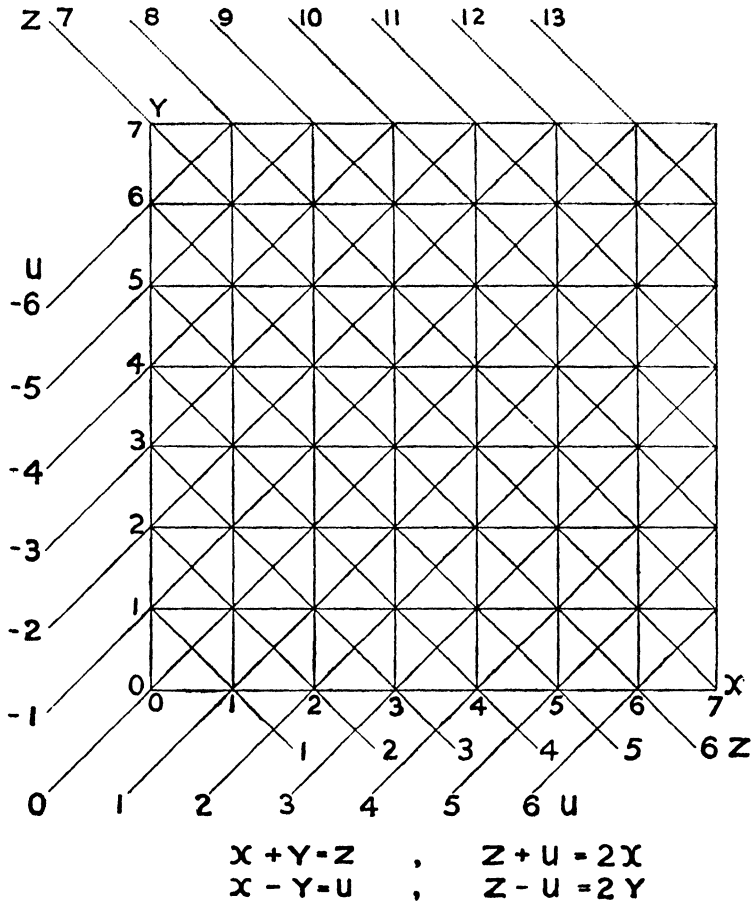


FIG. 10.

On such a figure it is, in general, possible, when values of two variables are given, to obtain those of the others. Thus for Fig. 10, given $z = 7$ and $u = 1$, we read $x = 4$ and $y = 3$. This depends

upon the fact that the position of a point in a plane is determined by two co-ordinates, while on such a nomogram every point has several co-ordinates.

We may now pass from this trivial example to the analogous but more difficult problem of the nomographic description of the phenomena discussed in the preceding lecture.

The construction of a complete nomogram for blood may be conveniently begun with the help of the oxygen and carbon dioxide dissociation curves. For the present purpose we have at our disposal the oxygen dissociation curves of the blood of A.V.B. at 3, 20, 40, and 80 mm. of CO_2 tensions, respectively (Fig. 4). These have been constructed with the

TABLE I

EFFECT OF CO_2 TENSION ON OXYGEN CURVES OF HUMAN BLOOD

O_2 tension.	HbO_2 Oxygen saturation of blood at following CO_2 tensions.			
	CO_2 = 3 mm.	CO_2 = 20 mm.	CO_2 = 40 mm.	CO_2 = 80 mm.
mm.	*saturation per cent*	*saturation per cent*	*saturation per cent*	*saturation per cent*
5	13·5	6·8	5·5	3·0
10	38·0	19·5	15·0	8·0
20	77·6	50·0	39·0	26·0
30	92·0	72·2	60·6	44·8
40	96·7	87·0	76·0	63·5
50	98·5	93·3	85·5	76·9
60	100	96·3	90·5	85·0
70	100	98·0	94·0	90·3
80	100	99+	96·0	93·7
90	100	100	97·5	95·7
100	100	100	98·6	97·1

aid of more than 90 experimental determinations. The curves yield Table I.

We also possess carbon dioxide dissociation curves

for the reduced and oxygenated blood of A.V.B. drawn with the aid of about 60 experimental

TABLE II

CO_2 Absorption Curves of Oxygenated and Reduced Human Blood

CO_2 tension.	Total CO_2 content.	
	Oxygenated blood (HbO_2 = 100 per cent).	Reduced blood (HbO_2 = 0 per cent).
mm.	*vol. per cent* CO_2	*vol. per cent* CO_2.
3	14·0	19·5
10	26·8	32·5
20	36·5	42·4
30	43·0	49·1
40	48·0	54·3
50	52·2	58·6
60	56·2	62·5
70	59·7	65·9
80	63·0	69·1

determinations (Fig. 5). These curves yield Table II.

From Table II, Fig. 11 has been constructed. Here values of total oxygen are plotted as abscissæ, values of total carbonic acid as ordinates, while values of carbon dioxide tension appear as contour lines. These lines are almost exactly straight, because at constant hydrogen-ion concentration equal changes in oxygenation are accompanied by equal changes in base bound by hæmoglobin. The values of total oxygen have been spaced in units of percentage by volume (100 per cent HbO_2 = 20 volumes per cent) in order to obtain the same values for the scales of ordinates and of abscissæ.

The next step consists in applying the data of Table I to Fig. 11, as follows: Beginning with the

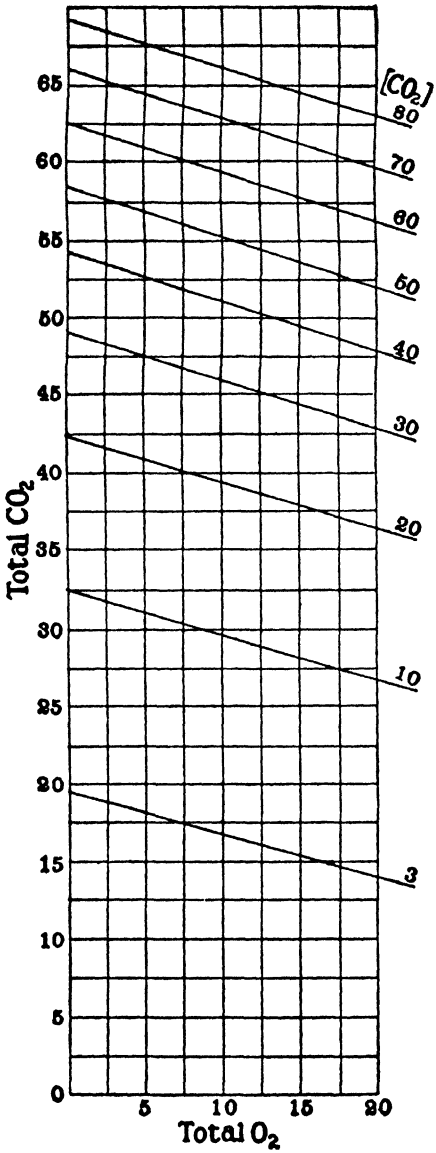


FIG. 11.—TRANSFORMATION OF CARBON DIOXIDE ABSORPTION CURVES OF BLOOD.

case $O_2 = 5$ mm., we select the pair of values $CO_2 = 3$ mm. and $HbO_2 = 13{\cdot}5$ per cent of complete saturation, or 2·7 volumes per cent of total O_2, 20 volumes per cent being assumed to represent 100 per cent saturation. We find the point on Fig. 11 where the abscissa corresponding to this value of total oxygen cuts the contour line corresponding to $CO_2 = 3$ mm. and mark the point. This process is then repeated for the other pairs of values of CO_2 tension and total oxygen corresponding to $O_2 = 5$ mm. and the four points are joined by a smooth curve. This curve is the contour line for $O_2 = 5$ mm. and is so marked. The process is now

repeated in turn for $O_2 = 10, 20, \ldots$ 100 mm., with the result represented by Fig. 12.

This is a Cartesian nomogram which completely illustrates the conditions of equilibrium between free and total oxygen and free and total carbonic acid, incompletely expressed by the dissociation curves. Because the Cartesian co-ordinates are values of total oxygen and total carbonic acid, respectively, while the contour lines represent the true physiological variables, it is in some respects the most useful of all representations of the blood equilibrium. These advantages will appear in the sequel.

At this stage it will

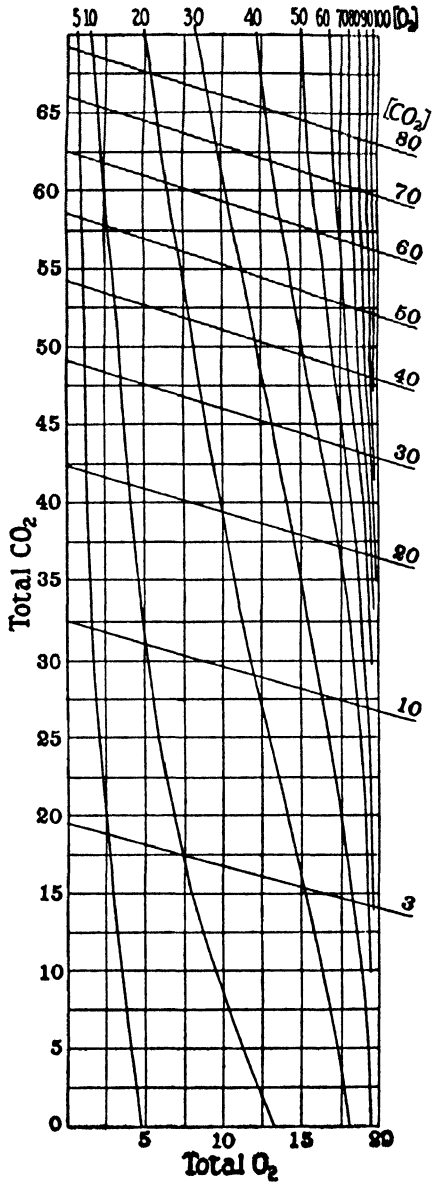


FIG. 12.—ABSORPTION OF OXYGEN AND CARBON DIOXIDE MIXTURES BY BLOOD.

be convenient to undertake a transformation to an alignment chart, or nomogram, of the type invented by d'Ocagne. The necessary construction for such a transformation (Fig. 13) is as follows:

Let *Ox* and *Oy* be the axes of a Cartesian nomogram and *KL* any straight line. Draw two parallel axes *Au* and *Bv*. Now read the Cartesian co-

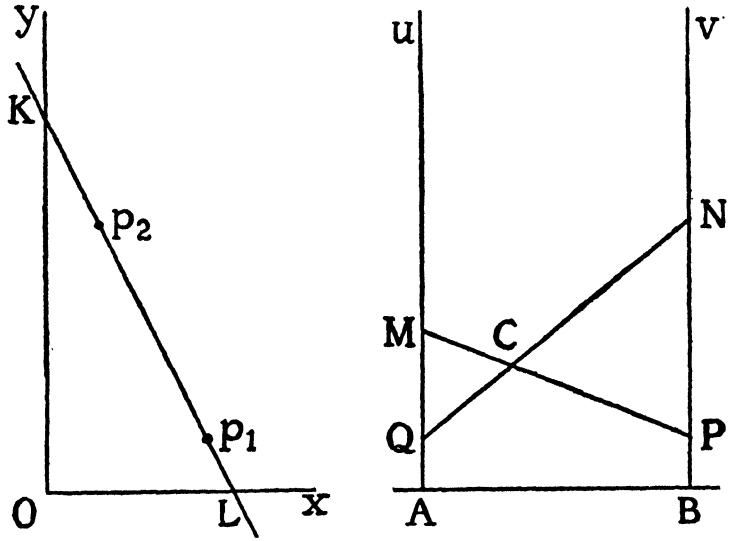


FIG. 13.—CONSTRUCTION OF AN ALIGNMENT CHART.

ordinates, x_1, y_1, and x_2, y_2, of any two points, say p_1 and p_2, of the line *KL*. On *Au* lay off (taking account of sign) the distance $AM = x_1$ and on *Bv* the distance $BP = y_1$. Join *MP*. On *Au* lay off the distance $AQ = x_2$ and on *Bv* the distance $BN = y_2$. Join *NQ*. Then *C*, the point of intersection of *MP* and *NQ*, is the correlative of the line *KL*. If *KL* is one of several contour lines, points corresponding to the others may be found by the

same method, and, upon joining all these points, a scale of values of the variable, z, corresponding to the contour lines, is obtained.

This process may be repeated for any other family of contour lines, corresponding to values of any other variable, w, on the same Cartesian background. Graduation of Au and of Bv to represent values of the variables x and y completes the construction.

The chart is read with the help of a thread stretched across the scales. It has the property that the values intercepted on the scales by any straight line are simultaneous values of the several variables. This is obvious, because, on such a chart, a straight line corresponds to a point on a Cartesian nomogram. Therefore the intercepts of the line on the scales correspond to the values of the variables represented by the scales at the point of the Cartesian nomogram which is the correlative of the straight line in question.

D'Ocagne's method has been widely applied and many expositions of the subject are now available. For further information his own treatise or that of Lipka may be consulted.

It is easy to see that this method is only applicable to Cartesian nomograms on which the contour lines are straight or may be so regarded without serious error. In the present case, however, the curvature of the contour lines of oxygen tension is considerable. Nevertheless, by confining our attention to the region of the chart where the CO_2 tensions fall within the physiological range, it is possible to replace the oxygen contour curves by straight lines, without

introducing serious error. We may then proceed to the transformation. When completed this results in the four scales marked HbO_2, O_2, CO_2, and total CO_2 of Fig. 14.

The scales marked *Vol.*, *r*, and pH_s complete the

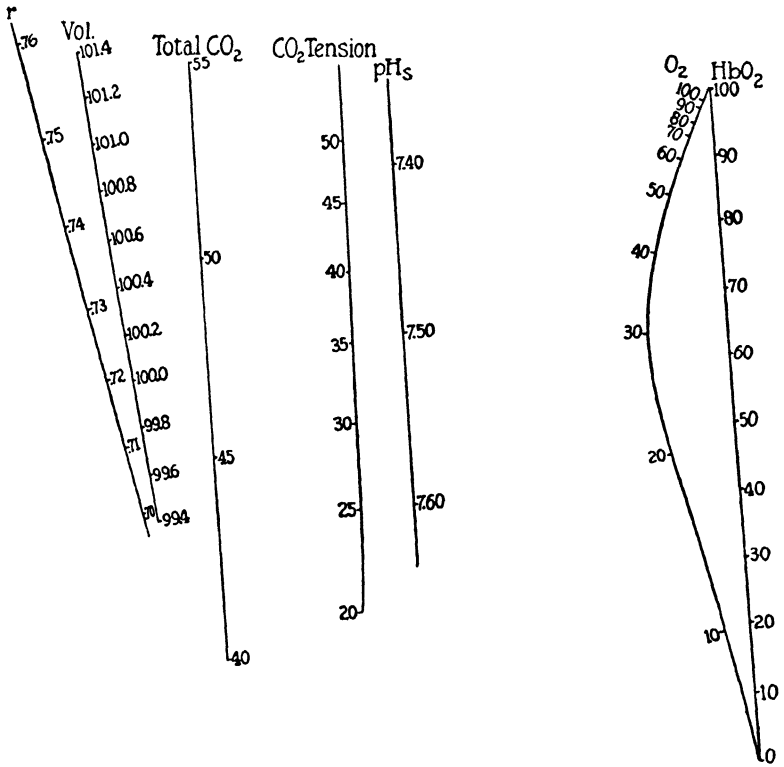


FIG. 14.—ALIGNMENT CHART FOR BLOOD OF A.V.B.

nomogram. For their construction further experimental data were required. These consist of: (1) measurements of the total CO_2 content of true plasma of specimens of blood of A.V.B., in which the O_2 tensions, total CO_2, and CO_2 tensions of the whole

blood were known; and (2) measurements of the indices of refraction of samples of true plasma of the blood of A.V.B. for which O_2 and CO_2 tensions were known.

From the determinations of indices of refraction of serum, after slight corrections for fluctuations from day to day, the volumes of the corpuscles were calculated. These volumes were expressed as percentages of the cell volume when $O_2 = 80$ mm. and $CO_2 = 39$ mm., which was found by numerous hæmatocrit readings to average 40 per cent of the total blood volume. At this point cell water constituted 65 per cent of total cell volume and serum water 91·5 per cent of total serum volume. This calculation calls for no comment, since, for the conditions of the experiment, index of refraction varies inversely with water content. The calculated values of cell volume were then applied to Fig. 11, and, taking account of the theoretical relationships developed by Van Slyke, Wu and McLean, straight lines were drawn to represent the best fitting values of cell volume. These lines were then used for the construction of the scale marked *Vol.* upon Fig. 14.

Next these values of *Vol.* and the data for total CO_2 in whole blood and true plasma were used to calculate the concentrations per litre of water of combined carbonic acid in cells and plasma, respectively. From these results values of the Donnan ratio:

$$r = \frac{[BHCO_3]_c}{[BHCO_3]_s} = \frac{[\bar{HCO_3}]_c}{[\bar{HCO_3}]_s} = \frac{[\bar{Cl}]_c}{[\bar{Cl}]_s} = \frac{[\bar{A}]_c}{[\bar{A}]_s} = \frac{[\bar{OH}]_c}{[\bar{OH}]_s} = \frac{[\overset{+}{H}]_s}{[\overset{+}{H}]_c}$$

were calculated. Finally, values of pH_s were obtained by means of the equation:

$$pH_s = 6{\cdot}12 - \log \frac{[H_2CO_3]_s}{[BHCO_3]^s}$$

The values of r and pH_s were then fitted as accurately as possible with straight lines on Fig. 11, and these lines were transformed into scales on Fig. 14.

From this nomogram, Fig. 14, it is possible to read directly, or to deduce by simple computation, the magnitudes of all known phenomena of the respiratory mechanism in blood.

It should be clearly understood that Fig. 14 is the quantitative description of a particular specimen of blood, closely corresponding in composition to the average composition of the blood of A.V.B. during a period of rather more than a year. For other specimens of human blood, for the blood of other species, and for the blood of various pathological states, changes occur in the nomogram. Such changes, however, affect only the magnitudes, and, in a lesser degree, the minor peculiarities of the disposition of the scales. It now seems clear that a really considerable change in the nomogram is out of the question.

The conclusion seems justified that the *general form* of the nomogram, Fig. 14, represents what may properly be called the law of the blood. With the consideration of differences from individual to individual, from normal to pathological, and from

species to species, we enter a field in which the comparative biological method becomes quantitative and rational.

On Fig. 14 seven variables are explicitly represented. A large number of others are, however, implicitly defined and may be deduced with the help of very simple calculations. Thus, taking account of the definition of r given by the equation:

$$r = \frac{[\overset{+}{\mathrm{H}}]_s}{[\overset{+}{\mathrm{H}}]_c}$$

we may use the scales of r and of pH_s to obtain values of pH_c. In order to avoid the necessity of making such calculations, it is more convenient to construct the scale of pH_c. This may be done as follows: Choose any value of pH_c, say 7·30, and any convenient pair of values of pH_s, 7·42 and 7·45. Now substitute these values in the equation:

$$\log r = pH_c - pH_s$$

obtained from the above equation by taking logarithms and putting $pH = -\log [\overset{+}{\mathrm{H}}]$.

$$\log r_1 = 7{\cdot}30 - 7{\cdot}42$$
$$\log r_2 = 7{\cdot}30 - 7{\cdot}45$$
$$r_1 = 0{\cdot}76$$
$$r_2 = 0{\cdot}71$$

Then the intersection of the lines joining $r = 0{\cdot}76$ with $pH_s = 7{\cdot}42$ and $r = 0{\cdot}71$ with $pH_s = 7{\cdot}45$ is the point corresponding to $pH_c = 7{\cdot}30$. In like manner such other points on the pH_c scale as may

be necessary to construct the scale are readily found. This construction has been performed and the result reproduced on the large-scale nomogram, Fig. 15. Here, too, are represented a number of other variables whose values have been obtained by equally obvious computations and analogous constructions.

It will perhaps suffice to define the variables represented by the several scales of Fig. 15. Taken in order from left to right they are as follows:

I. $(Cl)_s$. The concentration of serum chloride, expressed in millimols per litre of serum.

II. *r*. Donnan's

$$r = \frac{[BHCO_3]_c}{[BHCO_3]_s} = \frac{[\bar{HCO_3}]_c}{[\bar{HCO_3}]_s} = \frac{[\bar{Cl}]_c}{[\bar{Cl}]_s} = \frac{[\bar{A}]_c}{[\bar{A}]_s} = \frac{[\bar{OH}]_c}{[\bar{OH}]_s} = \frac{[\overset{+}{H}]_s}{[\overset{+}{H}]_c}$$

Here the brackets represent concentrations per litre of water of serum or cells, as the case may be.

III. $(Cl)_c$. The concentration of cell chloride, expressed as millimols per litre of cells.

IV *a*. Δ (Cl). The difference, expressed as millimols per litre of blood, compared with arterial blood, in the total amount of chlorides in cells or serum.

IV *b*. Per cent A in cells. The percentage of total blood chloride or bicarbonate present in the cells.

V *a*. *V* of cells. The volume of the cells, expressed as percentage of this volume at about $O_2 = 80$ mm. and $CO_2 = 39$ mm., the point where this volume is 40 per cent of the total blood volume.

V *b*. Per cent H_2O in cells. The percentage of total blood water present in the cells.

VI. BP_c. The base combined with cell protein, expressed as millimols of base per litre of blood.

VII. Cell $BHCO_3$. The combined carbonic acid of the cells per litre of blood, expressed: (*a*) as millimols, and (*b*) as volumes per cent.

VIII. $(BHCO_3)_c$. The combined carbonic acid of the cells per litre of cells, expressed: (*a*) as millimols per litre, and (*b*) as volumes per cent.

IX. $(BHCO_3)_B$. The combined carbonic acid of whole blood, expressed: (*a*) as millimols per litre, and (*b*) as volumes per cent.

X. $(BHCO_3)_s$. The combined carbonic acid of serum per litre of serum, expressed: (*a*) as millimols per litre, and (*b*) as volumes per cent.

XI. Serum $(BHCO_3)$. The combined carbonic acid of serum per litre of blood, expressed: (*a*) as millimols per litre, and (*b*) as volumes per cent.

XII. Total CO_2. The total carbonic acid of blood, expressed: (*a*) as millimols per litre, and (*b*) as volumes per cent.

XIII. Δc. The change in concentration of total solute, compared with arterial blood, expressed in millimols per litre of blood.

XIV. CO_2. Free carbonic acid, expressed: (*a*) as millimols per litre of blood, and (*b*) as millimetres of partial pressure of mercury.

XV *a*. BP_s. Base bound by protein of serum, expressed as millimols of base per litre of blood.

XV *b*. pH_s. $-\log [\overset{+}{H}]$ in serum.

XVI. pH_c. $-\log [\overset{+}{H}]$ in cells.

XVII. O_2. Oxygen tension, expressed as millimetres of partial pressure of mercury.

XVIII. HbO_2. Combined oxygen, expressed: (*a*) as millimols per litre of blood, and (*b*) as per cent of saturation. The scale for total oxygen is so nearly identical with this as to be practically indistinguishable from it.

Across the nomogram two straight lines are drawn. These lines define the equilibria of arterial blood and of venous blood, respectively.

The first is approximately true for all ordinary normal conditions; the second defines the conditions during moderate exercise. The graduated curves tangent to these lines near their point of intersection define the respiratory cycle. They will be discussed below in Lecture III.

It is important to note, as an aid in constructing similar nomograms from scanty data, that the point of intersection of arterial and venous lines depends upon the respiratory quotient. On the nomogram broken lines mark the position of the points of intersection of arterial and venous lines corresponding to values of the respiratory quotient in the range between 0·70 and 1·00.

The nomogram may now be used as a means of discovering the respiratory changes of A.V.B. Tables III, IV, and V contain the values of the several quantities as read from Fig. 15.

Table III reveals one fact which merits explicit mention. Reference to the values of $BHCO_3$ shows that in the passage through the lungs plasma yields

TABLE III

Blood

Concentration of hæmoglobin = 8·8 mM. per litre blood.
„ „ serum proteins = 51 gm. „ „
Respiratory quotient = 0·82.

		Arterial.			Venous.			Δ		
		Serum.	Cells.	Whole blood.	Serum.	Cells.	Whole blood.	Serum.	Cells.	Whole blood.
H_2O	*cc. per l. blood*	549	260	809	544	265	809	− 5	+ 5	0·0
B	*mM.* „ „	84·02	48·32	132·34	84·00	48·34	132·34	0·0	0·0	0·0
Cl	„ „ „	59·59	20·41	80·00	58·45	21·55	80·00	− 1·13	+ 1·13	0·0
BP	„ „ „	9·20	22·70	31·90	9·09	20·73	29·82	− 0·11	− 1·97	− 2·08
$BHCO_3$	„ „ „	15·23	5·21	20·44	16·46	6·06	22·52	+ 1·23	+ 0·85	+ 2·08
H_2CO_3	„ „ „	0·71	0·34	1·05	0·82	0·40	1·22	+ 0·11	+ 0·06	+ 0·17
Total CO_2	„ „ „	15·94	5·55	21·50	17·28	6·46	23·75	+ 1·34	+ 0·91	+ 2·25
Free O_2	„ „ „	—	—	0·07	—	—	0·03	—	—	− 0·04
Combined O_2	„ „ „	—	8·5	8·5	—	5·8	5·8	—	− 2·7	− 2·7
Total O_2	„ „ „	—	—	8·6	—	—	5·8	—	—	− 2·7
CO_2 tension, *mm. Hg*		—	—	40	—	—	47	—	—	+ 7
O_2 tension	„	—	—	78	—	—	34	—	—	− 44
Volume, *c.c. per l. blood*		599·7	400·3	1,000	595·0	405·0	1,000	− 4·7	+ 4·7	0·0
pH		7·450	7·309	—	7·421	7·300	—	− 0·03	− 0·01	—
$r = \frac{[H]_e}{[H]_c} = \frac{[A]_e}{[A]_c}$		—	—	0·7215	—	—	0·7576	—	—	0·036
Per cent A in cells		—	—	25·49	—	—	26·90	—	—	1·4
Total concentration *mM.* per *l*		—	—	—	—	—	—	—	—	2·2

TABLE IV

SERUM

		Arterial.	Venous.	Δ
H_2O	*c.c. per l. serum* . .	915·4	914·3	− 1·0
B	*mM.* „ „ . .	140·1	141·1	+ 1·0
Cl	„ „ „ . .	99·37	98·22	− 1·15
BP	„ „ „ . .	15·34	15·27	− 0·07
$BHCO_3$	„ „ „ . .	25·40	27·66	+ 2·26
H_2CO_3	„ „ „ . .	1·17	1·38	+ 0·21
Total CO_2	„ „ „ . .	26·58	29·04	+ 2·46

TABLE V

CELLS

		Arterial.	Venous.	Δ
H_2O	*c.c. per l. cells* . .	649·5	654·2	+ 5
B	*mM.* „ „ . .	120·7	119·3	− 1·4
Cl	„ „ . .	50·98	53·21	+ 2·23
BP	„ „ . .	56·70	51·18	− 5·52
$BHCO_3$	„ „ . .	12·99	14·96	+ 1·97
H_2CO_3	„ „ . .	0·85	0·99	+ 0·14
Total CO_2	„ „ . .	13·86	15·95	+ 2·09
Combined O_2	„ „ . .	21·2	14·3	− 6·9

60 per cent and cells only 40 per cent of the total amount of combined carbonic acid eliminated. Now this escape of combined carbonic acid depends upon the reaction, $BHCO_3 + HP = BP + H_2CO_3$, and, as the table also shows, cell protein, or essentially hæmoglobin, is responsible for 95 per cent of this reaction. Thus it appears that hæmoglobin is responsible, under these conditions, for the *transport* of more than 90 per cent of all the carbonic acid excreted. The fact is masked by the accompanying

redistribution of chloride ions. Moreover, less than 10 per cent of this 90 per cent is due to buffer action of hæmoglobin, and, accordingly, it may be concluded that about 80 per cent of all carbonic acid excreted is dependent for its transport, under normal conditions, upon the oxygen effect upon the acidity of the hæmoglobin molecule. In short, hæmoglobin is, though indirectly, hardly less important in the transport of carbonic acid than in that of oxygen itself.

This is one aspect of the phenomenon. Another, hardly less important, is the influence of hæmoglobin in stabilising the hydrogen-ion concentration of the plasma. The actual difference in reaction of plasma between arterial and venous blood, as shown in Fig. 15, is about 7 per cent of the concentration of hydrogen and hydroxyl ions. In the absence of hæmoglobin this difference would be about tenfold larger. Given the buffer action of hæmoglobin, in the absence of the change in the hæmoglobin molecule accompanying change in oxygenation, it would be about threefold larger.

Thus, it may be seen that, during the respiratory cycle, hæmoglobin is of the greatest importance in stabilising the alkalinity of the blood, both through its protein nature and also because of the oxygen effect.

This single substance is indeed a veritable organ, in the physiological sense, performing three important functions with the utmost perfection. However, it owes its efficiency, not merely to its own

extraordinary physico-chemical properties, but also to those characteristics of the physico-chemical system in which it occurs and of which it is a component, that are described by Fig. 14. Each component of the system is, in fact, adjusted to all the others in a manner which well illustrates Claude Bernard's theories concerning organisation. Indeed, it is not too much to say that Fig. 15 is a quantitative mathematical description of an organised physico-chemical system. It also contains a complete description of the mechanism whereby the more important physico-chemical properties of the plasma are maintained nearly constant during the respiratory changes.

APPENDIX

Fig. 15 may be used to define any other conditions of equilibrium within the common ranges of arterial and mixed venous blood. To this end it will be found a very powerful instrument. Indeed an alignment chart is probably the only means of presenting such a great mass of quantitative information in compact form. But it must not be forgotten that most of the scales represent mathematical functions of a quite secondary character and that seven scales suffice completely to define the system of the blood in accordance with our present knowledge. Given these seven scales, all the others may be deduced without the use of any information beyond that involved in the construction of these fundamental scales.

Fig. 14 (or Fig. 15) may also be used to reconstruct Figs. 4 and 5 or to obtain any other contour line charts of a similar nature. As we have seen, there are, among seven variables, taking three at a time, 35 combinations. Moreover, in each of these 35 cases, three variables being

involved, it is possible to construct three contour line charts, taking in turn the first and second, the first and third, and the second and third variables as Cartesian ordinates. Thus a complete treatment involves the construction of 105 Cartesian contour line charts.

These 105 charts fall into 21 sets of 5 each. There are, in fact, 21 combinations, taking 2 at a time, among 7 variables. Therefore, there are 21 pairs of Cartesian co-ordinates. When 2 of the variables have been chosen as Cartesian co-ordinates, 5 remain. Accordingly, they yield 5 families of contour lines. Evidently the 5 members of each of these 21 sets of contour line charts form by superposition a Cartesian nomogram, which is the complete expression of the equilibrium and the equivalent in all respects of Fig. 14.

The construction of any one of these charts may be explained as follows: Let the variables in question be x, y, and z, represented by the scales u, v, and w, of Fig. 14, and let it be required to draw contour lines representing values of z on a Cartesian background of x and y. Choose suitable values for the z contour lines and find on w the points corresponding to these values. Through each of these points pass several straight lines and read the pairs of values of x and y defined by the intercepts of these lines on u and v. After tabulating the data thus obtained, it only remains to transfer them to a Cartesian background of x and y, and to join each set of points corresponding to each of the values of z.

These 105 charts may be found in the paper of Henderson, Bock, Field and Stoddard (8).

LECTURE III

DEDUCTIONS CONCERNING THE CIRCULATION

THE synthetic description of blood may now be used in order deductively to establish certain facts concerning the circulation. It is, indeed, clear that the properties of bloods must impose certain conditions upon the activity of the circulatory apparatus.

In Fig. 16 the abscissæ are values of total oxygen, the ordinates, values of total CO_2, while values of oxygen tension and CO_2 tension appear as contour lines. Fundamentally, therefore, the figure is a large-scale drawing of a portion of Fig. 12. Three points are marked on the figure: *L*, corresponding to the O_2 and CO_2 tensions of alveolar air, as directly determined; *A*, the arterial blood point, determined by repeated analyses of blood drawn from the radial artery; and *V*, the venous blood point. The position of this point is less accurately known. It must, however, fall somewhere on the line marked $RQ = 0{\cdot}81$, since this was the value of A.V.B.'s respiratory quotient for the conditions now under consideration, and it is evident that on this diagram any straight line drawn through the arterial point is a respiratory quotient line. The slope of the line is the measure of the respiratory quotient. Therefore, it is necessary to know only one fact regarding

the mixed venous blood in order to determine the position of the venous point on the diagram.

On the figure the position of the venous point has been chosen at $CO_2 = 47$ mm., a condition

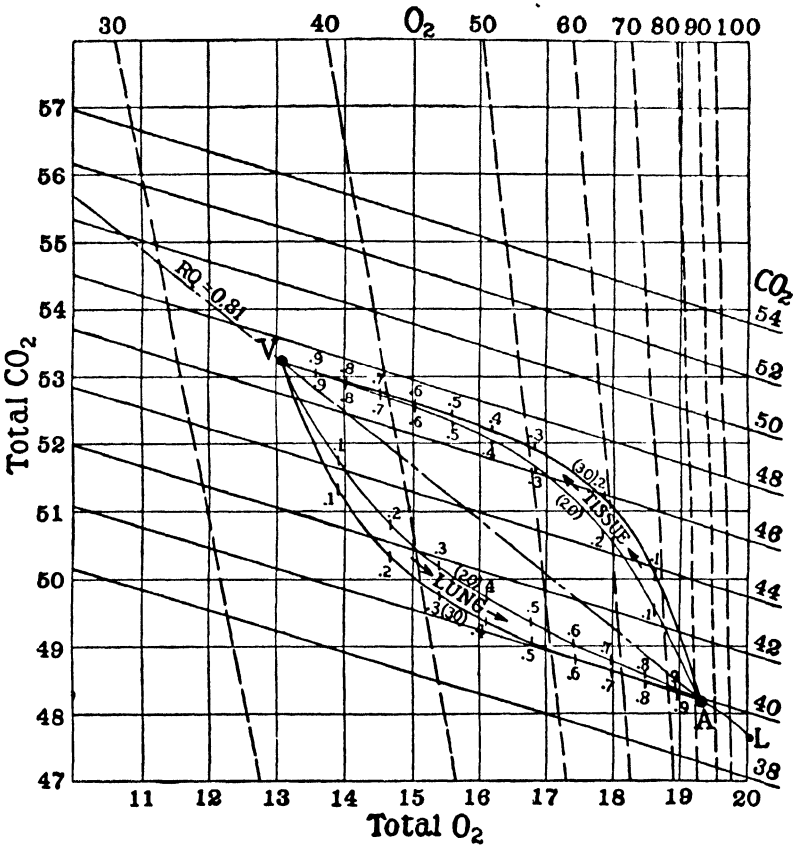


FIG. 16.—THE RESPIRATORY CYCLE.

which corresponds to a steady state during moderate exercise.

With the Cartesian co-ordinates, the O_2 and CO_2 contour lines, and the points *L*, *A*, and *V*, once established, it is possible to take another step.

The rate of increase of the oxygen content of an infinitesimal portion of a capillary column of blood in the lungs must be proportional to the difference between the alveolar oxygen tension, $[O_2]_L$, and that of the blood, $[O_2]_p$:

$$\frac{d(\text{total } O_2)}{dt} = a_1([O_2]_L - [O_2]_p) = a_1\Delta O_2$$

Similarly, $$\frac{d(\text{total } CO_2)}{dt} = a_2([CO_2]_L - [CO_2]_p) = a_2\Delta CO_2$$

Dividing, $$\frac{(d\text{total } CO_2)}{d(\text{total } O_2)} = \frac{a_2}{a_1} \times \frac{\Delta CO_2}{\Delta O_2}$$

The value of the constant term $\frac{a_2}{a_1}$ is not accurately known. For water its value is about 20, for the tissues about 30. But we are here concerned with conditions which are hard to define, since, to mention only one complication, the amount of mixing within the capillary, and hence the extent to which the exchanges between red cells and plasma are adjusted, remain unknown. We shall therefore make no attempt to estimate the value of $\frac{a_2}{a_1}$, but shall employ in turn the round values of 20 and 30 in order to discover, if possible, the general characteristics of the diffusion process.

For Fig. 16

$$\frac{d(\text{total } CO_2)}{d(\text{total } O_2)} = \frac{dy}{dx}$$

Therefore, it is evident that all points such that

$$\frac{a_2}{a_1} \times \frac{\Delta CO_2}{\Delta O_2} = m = \text{a negative constant}$$

or in words such that the difference between the CO_2 tension at the point and the CO_2 tension of the alveolar air, divided by the difference between the O_2 tension at the point and the O_2 tension of alveolar air is constant, are points which define a slope on the Cartesian co-ordinates.

The meaning of this slope may be easily understood from the following considerations. Instead of speaking of a point on the chart as defining a given condition of the blood, we may speak of the blood as existing at a point on the chart. Then, in order to reach the arterial point the blood may be said to describe a curve upon the chart. Now the direction in which the blood must be moving when at the point p is that of the slope, m_p, in question. In other words, this slope is the slope of the tangent, at the point p, to the curve over which the blood must pass as a result of a diffusion process. This is true because, as already explained, when the blood is at the point p,

$$\frac{dy}{dx} = \frac{d(\text{total } CO_2)}{d(\text{total } O_2)} = \frac{a_2}{a_1} \times \frac{([CO_2]_L - [CO_2]_p)}{([O_2]_L - [O_2]_p} = \frac{a_2}{a_1} \times \frac{(\Delta CO_2)_p}{(\Delta O_2)_p} = m_p$$

It is, accordingly, convenient to draw a family of contour lines, each one such that for every point of the contour line

$$\frac{a_2}{a_1} \times \frac{([CO_2]_L - [CO_2]_p)}{([O_2]_L - [O_2]_p)} = \frac{a_2}{a_1} \times \frac{(\Delta CO_2)_p}{(\Delta O_2)_p} = m_p = \text{a constant}$$

Here $[CO_2]_p$ and $[O_2]_p$ are the CO_2 tension and O_2 tension corresponding to any point, p, of the contour line and $[CO_2]_L$ and $[O_2]_L$ the CO_2 tension and the O_2 tension corresponding to the alveolar air point, L.

Next, taking $\frac{a_2}{a_1} = 20$, the characteristic slope, $\frac{dy}{dx} = \frac{d(\text{total } CO_2)}{d(\text{total } O_2)}$, defined by each contour line, is calculated, and a large number of short parallel lines of the calculated slope, each intersecting the contour line, are drawn. It now remains to join the point V and the point L by means of a curve which cuts each of these contour lines so that the tangent to the curve at each point of intersection with a contour line is parallel with the characteristic slope defined by the contour line. The curve thus constructed is the required representation of the diffusion process in the lung.

Taking $\frac{a_2}{a_1} = 30$, a similar curve is obtained. These two curves are represented on Fig. 16 and are marked "LUNG (20)" and "(LUNG 30)."

The analogous curves for the tissue diffusion process, assuming a condition in which the local venous blood is of the same composition as the mixed venous blood, are somewhat more difficult to obtain and also more uncertain. This depends upon the fact that, in the absence of information concerning O_2 and CO_2 tensions within the tissues, it is necessary to proceed by a method of successive approximations. Thus have been obtained the curves marked "TISSUE (20)" and "TISSUE (30)" on Fig. 16.

The researches of Krogh justify the belief that the outer curves "LUNG (30)" and "TISSUE (30)"

more nearly represent the process as it might take place under ideal conditions. But those peculiarities of the blood which are responsible for the wide separation of the diffusion curve for the lung from that for the tissue are in part dependent upon heterogeneous reactions between cells and plasma. Therefore, taking account of the uncertainty regarding the completeness of such reactions during the passage of blood through capillaries, we shall employ the curves (20). In so doing we wish merely to imply that the differences between the diffusion process in the lung and the reverse process in the tissues are probably *at least* as great as these two curves indicate. In any event, it is evident that the cycle marked (20) and that marked (30) are not very unlike. They are, in fact, necessarily very much alike in all but magnitude, i.e. in radius of curvature.

On the large alignment chart (Fig. 15) two curves are drawn. These are the envelopes of all lines corresponding to points on the cycle (20) of Fig. 16. Every tangent to one or the other of these curves represents some point on this cycle. It is therefore possible, with the help of Fig. 15, thus modified, to discover the sequence of simultaneous changes in all the variables during the respiratory cycle. This description applies to that case only when the composition of arterial blood and that of venous blood are represented by the points A and V. For any other pair of arterial and venous points integration will yield another cycle.

It is now possible to proceed to further results.

In order to fix our ideas we may begin with a tabulation of simultaneous values of the concentrations of free oxygen and of oxyhæmoglobin, throughout the cycle represented by Fig. 16.

TABLE VI

HbO_2	pO_2 mm.	
saturation per cent	Lung.	Tissues.
65	34·0	34·0
70	37·0	37·4
75	40·5	41·3
80	44·7	45·7
85	50·0	51·4
90	58·0	61·2
95	73·2	75·0
96	78·0	78·0

Taking first the case of the lung, it is evident that the above table makes possible the calculation, for every value of HbO_2, of the effective head of oxygen pressure causing the diffusion of oxygen from alveolar air to blood. This head of pressure is, in fact, equal to the mean partial pressure of oxygen in the alveolar

TABLE VII

LUNG

HbO_2	O_2 head (Δp)
saturation per cent	*mm.*
65	76·0
70	73·0
75	69·5
80	65·3
85	60·0
90	52·0
95	36·8
96	32·0

air, minus the concentration of free oxygen in blood, expressed as millimetres of mercury. Thus it is easy by simple subtraction, given the partial pressure of oxygen in the alveolar air of A.V.B. as 110 mm., to obtain the values of Table VII.

Making use of this table, we may next lay off as ordinates on Fig. 17 values of HbO_2, and at convenient intervals draw sets of parallel lines whose slopes measure the head of oxygen pressure for the ordinates on which they are placed. Then that curve which, beginning at $HbO_2 = 66$ per cent and ending at $HbO_2 = 96$ per cent, is everywhere parallel, at the corresponding values of HbO_2, with the slopes thus drawn, represents the necessary course of the diffusion process in the lung. Therefore, if we assume uniformity of structure and of blood flow in the capillary, the abscissa under the curve may be taken to represent the length of an average lung capillary, or, what comes to the same thing, time.

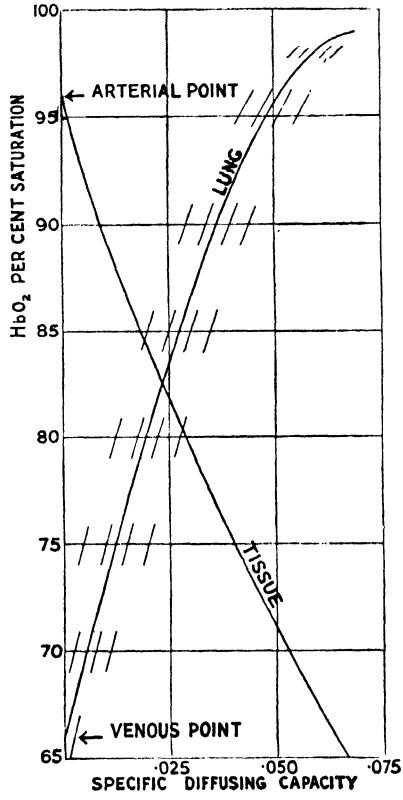


FIG. 17.—GRAPHICAL ESTIMATION OF DIFFUSING CAPACITY.

Accordingly, it is only necessary to divide the abscissa under the curve into ten equal parts and to read from the curve the values of HbO_2 corresponding to these divisions, in order to be able to graduate the process of diffusion in the lung along the capillary, in time units.

On the assumption that the oxygen concentration in the tissues is negligibly small, and taking into consideration an average capillary, which delivers blood of the composition of mixed venous blood to the vein, it is easy to repeat the construction and thus to obtain a corresponding result for a capillary of the greater circulation. This result is also shown on Fig. 17. Here, again, one is dealing with an ideal capillary corresponding to a statistical mean, and in this case, no doubt, the extreme departures from the mean which actually occur in the organism are very large. It is also extremely improbable that in all parts of the body the oxygen concentration of the tissues should be negligibly small. We shall return to a consideration of these difficulties.

The graduations thus made possible have been marked on the cycles of Fig. 16 and on the envelopes of Fig. 15. With this addition the description of the two figures is complete.

A closer examination of the cycle is not without interest. Certain variables, indeed, notably the concentration of free oxygen and the hydrogen-ion concentration of the corpuscles, pass through quite characteristic series of changes. The cycles for these variables will be found in Figs. 18 and 19.

It is easy to see in the oxygen pressure–time cycle one more expression of the peculiarities of hæmoglobin. This calls for no further comment.

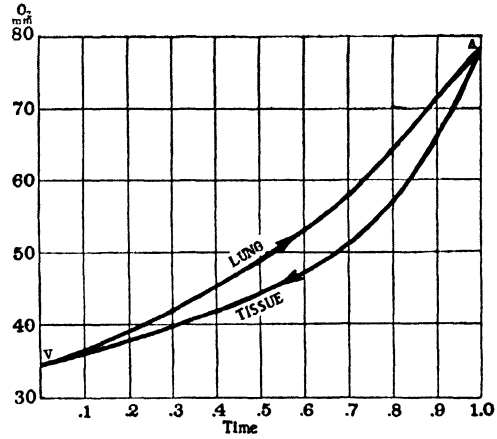


FIG. 18.—CHANGES OF OXYGEN PRESSURE IN BLOOD DURING THE RESPIRATORY CYCLE.

The case of the cycle of values of pH_c is even more curious, and somewhat less easy to grasp. Yet this, too, is, in the main, an expression of the properties of the hæmoglobin molecule. It will be seen that both in lung and in tissue capillaries the value of this variable promptly overshoots its mark, so to speak, and then gradually returns to its final value. Under certain circumstances such a phenomenon might be of great importance. For example, the hydrogen

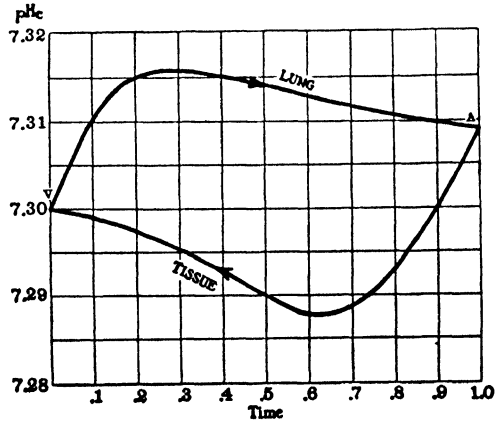


FIG. 19.—CHANGES OF HYDROGEN-ION CONCENTRATION OF THE CORPUSCLES DURING THE RESPIRATORY CYCLE.

ion might exercise a "trigger action" at values of pH greater than that of arterial blood. Then such action would be possible in this case, in spite of its apparent impossibility as judged by the concentration of the hydrogen ion in arterial and venous blood.

From what has been said above it is evident that we may draw useful conclusions from a comparison of the lengths of the abscissæ under the curves of Fig. 17. These lengths are, in fact, proportional to the total areas of uniform diffusing surface over which, in equal periods of time, equal volumes of arterial and venous blood must pass, under the conditions which have been assumed in the construction of the figures, in order that the respiratory exchange may be accomplished. Each length may be regarded as a measure of the *specific diffusing capacity* of the capillary system in question, in other words, of the diffusing capacity, per litre of blood flow, per minute, of the capillaries of the lesser and of the greater circulations respectively. Their ratio measures, therefore, the relative diffusing capacities of lung and tissue capillaries for the conditions now under discussion. We may draw the conclusion that in a normal individual, under conditions of moderate exercise, when about one-third of its oxygen content is removed from the blood in its passage through the greater circulation, the diffusing capacity of the active capillaries of the greater circulation must be at least 20 per cent greater than that of the active capillaries of the lung.

There is no difficulty in repeating this investigation for other cases. At present it will, perhaps, suffice to restrict our attention to three other instances

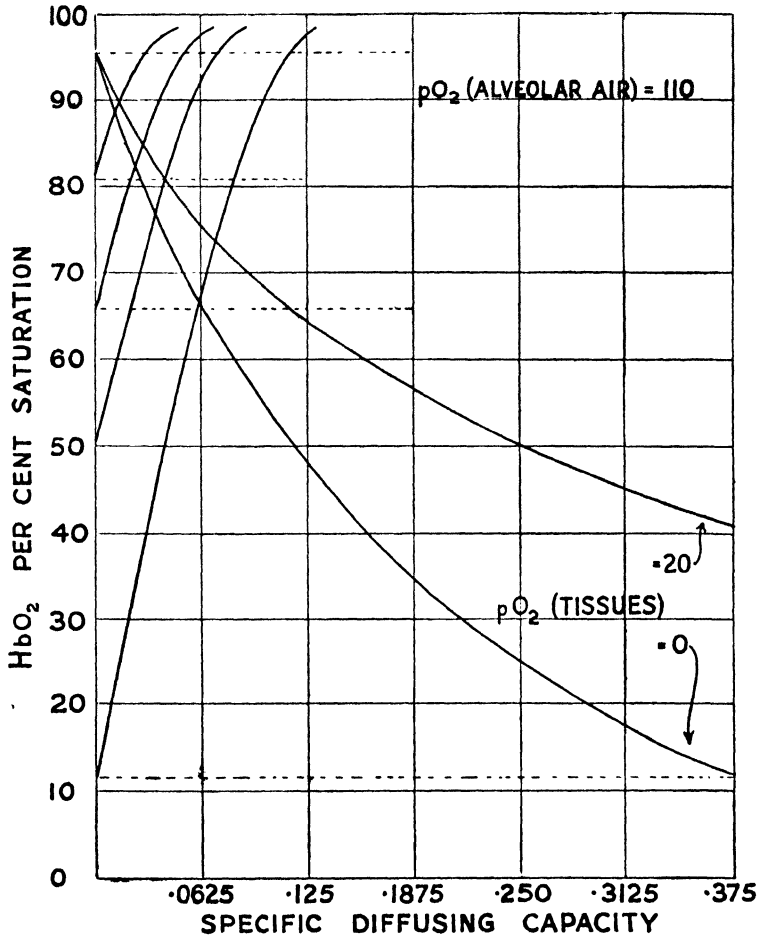


FIG. 20.—DIFFUSING CAPACITIES IN DIFFERENT RESPIRATORY CYCLES.

in which the arterial point as well as the respiratory quotient remain unchanged, while the venous point falls at 81, 50, and 11 per cent HbO_2, respectively. For each instance two cases have been considered:

(1) where pO_2 in the tissues is taken as zero; (2) where this value is taken as 20 mm. The result of repeating the constructions above described is Fig. 20, to which, for convenience, the curves of Fig. 17 have been added. The values of the abscissæ of the curves of Fig. 20 are assembled in Table VIII.

TABLE VIII

SPECIFIC DIFFUSING CAPACITY (ARTERIAL POINT = 96 % HbO_2)

Venous point . . .	81 %	66 %	50 %	11 %
Lung (pO_2 = 110) . .	0·030	0·051	0·072	0·114
Tissues (pO_2 = 0) . .	0·027	0·063	0·118	0·375
Tissues (pO_2 = 20) . .	0·038	0·120	0·250	—

More clearly to illustrate the conditions imposed upon the organism by the properties of the blood, the data of Table VIII have been converted into those of Table IX. Here are presented the values

TABLE IX

DIFFUSING CAPACITY PER LITRE OF OXYGEN PER MINUTE (ARTERIAL POINT = 96 % HbO_2)

Venous point . . .	81 %	66 %	50 %	11 %
ΔO_2 %	15 %	30 %	46 %	85 %
Lungs (pO_2 = 110) . .	1·00	0·85	0·79	0·67
Tissues (pO_2 = 0) . .	0·90	1·05	1·29	2·21
Tissues (pO_2 = 20) . .	1·27	2·00	2·74	—

of diffusing capacity, per litre of oxygen diffusing, per minute.

These tables demonstrate that the total area of diffusing surface, which we may assume to be roughly proportional, for similar structures, to the number of physiologically active capillaries, is subject

to wide variation. Such variation is the expression of a simple physical necessity. We are, fortunately, able to present a rough estimate of the magnitude of this variation in one instance.

In a series of experiments recently performed at the Massachusetts General Hospital, the blood of A.V.B., which has provided the data for all the above calculations, was found to be about 75 per cent saturated on its return to the heart during rest, and about 66 per cent saturated during moderate exercise on a stationary bicycle. The arterial blood meanwhile remained nearly unchanged at the value $HbO_2 = 96$ per cent. This exercise produced approximately a fourfold increase in the flow of blood per minute.

These conditions correspond to the following values of specific diffusing capacity :

	Rest.	Work.
Pulmonary circulation . .	0·040	0·051
Greater circulation . . .	0·040	0·063

In order to obtain values of the total diffusing capacity, these values must be multiplied by the volume of blood flow per minute. Thus, taking the blood flow at rest as 5 litres per minute, the total diffusing capacity appears to have, in arbitrary units, the following values :

	Rest.	Work.
Pulmonary circulation . . .	0·20	1·02
Greater circulation . . .	0·20	1·26

It seems clear that really hard work must be accompanied by a further large increase of total diffusing

capacity. Even moderate work, however, produces changes which are very striking. There seems no reason to doubt that the number of patent capillaries in the lung has increased in this experiment about fivefold, and, unless there has been a substantial fall in the tension of oxygen in the muscles, the number of patent capillaries in the greater circulation must have undergone a still larger increase.

The increased transport of oxygen has been accomplished in this case by what appears to be an increase in the diffusing capacity of the capillary system approximately proportional to the total oxygen consumption, by a large but slightly smaller increase in the flow of blood, and by a small increase in the coefficient of utilisation of oxygen. Indeed, it seems not impossible that in the active tissues the last of these variables has remained nearly constant. For we should expect that the blood returning from inactive parts of the body must contain more oxygen than that which has supplied active tissues, and it is clear that a larger fraction of the circulating blood passes through active tissues during exercise than during rest. Under these circumstances an increase of activity might well bring about a substantial increase in the coefficient of utilisation of oxygen for the whole body, although the coefficient for each organ and tissue remained substantially unchanged. Suppose, for example, that during rest 4 litres of the blood flows through inactive tissues which reduce the oxyhæmoglobin to 78 per cent, and 1 litre through active tissues which reduce the oxyhæmo-

globin to 63 per cent. If, then, during exercise the whole increase of blood flow, 15 additional litres, went to supply active tissues, and the coefficient of utilisation of active and inactive tissues remained unchanged, the result would be in agreement with the observations above reported. No doubt the real phenomenon is very far from this simple illustration, which is intended merely to define one element of a complex adjustment.

On more general grounds, there is reason to suppose that the capillary blood of a particular tissue is likely to remain relatively constant in composition, preserving more or less precisely its characteristic gradient from the beginning to the end of the capillary, at least during moderate changes of activity. Indeed, ever since the hypothesis of the constancy of the internal environment was first stated by Claude Bernard, there has been little but confirmation of his views, which, as we have seen, find particularly strong support in recent physico-chemical discoveries.

However this may be, it is clear, as above stated, that the greatly increased call for oxygen has been met in this instance by a very large increase in the capillary bed and in the volume of the blood flow, accompanied by no great change in the amount of reduction of the blood in its passage through the active regions of the body. We may now inquire what other changes might supply an equal amount of oxygen. The answer to this question may readily be found with the help of Table IX. According to

this table, the diffusing capacity necessary to permit the diffusion of a given amount of oxygen into the lung is less the greater the coefficient of utilisation. This must always be true if the arterial point remains nearly unchanged.

In the tissues, the greater the coefficient of utilisation, the greater is the diffusing capacity required for a given quantity of oxygen.[1] Thus it is apparent that it is ordinarily the conditions of diffusion in the tissues of the greater circulation, and, therefore, particularly in the muscles, which tend to associate a high degree of activity with a normal coefficient of utilisation of oxygen, and to bring about the adjustment above described. For example, in order that in the tissues arterial blood of 96 per cent HbO_2 may be reduced to venous blood of 11 per cent HbO_2, a diffusing capacity per litre of oxygen per minute of 2·21, instead of 1·05 for venous blood of 66 per cent HbO_2, would be required. This would lead, in the case of moderate exercise, to an increase of total diffusing capacity from the original value of 0·20 not merely to 1·26, but to 2·65. It is true that the blood flow would, under these circumstances, be increased only about 50 per cent (instead of fourfold) above the resting value. But this might be a very slight compensation. Indeed, it might well be the reverse, for the blood would, under these circumstances, be moving with only about one-tenth its normal velocity along each capillary, a condition on

[1] With constant venous point and varying arterial points these tendencies are reversed.

many other grounds hardly compatible with great activity.

Very different is the actual phenomenon, for in reality the blood is moving through each capillary, not only nearly unchanged in composition from its resting state, but also with nearly unchanged velocity, compared with the condition of rest.

This, in fact, is the concrete result of the present discussion, that a considerable increase in activity of an organ may be accompanied by the opening of capillaries approximately in the same proportion and by a like increase in the total flow of blood. Such a readjustment can involve but little change in the blood flow per capillary, and, therefore, in the composition of the blood and in the physico-chemical conditions of the environment of the cells.

In the light of these considerations the opening and closing of capillaries described by Krogh appear as one of the major physiological activities.

The conclusions which we have reached in this lecture, unlike the purely physico-chemical results considered in Lecture II, involve the consideration of a great variety of physiological phenomena. For this reason it is desirable to attach as little importance as possible to the estimates of magnitudes which have been involved. In particular it may be pointed out that errors of significant size may have been made in the estimates of the composition of mixed venous blood and of the blood flow. The oxygen and carbonic acid concentrations in the tissues are also uncertain, and it is impossible to estimate precisely

the magnitude of the effect of great differences in the composition of venous blood from different parts of the body upon our calculations. Finally, there is a problem concerning the pulmonary exchange which is at present the subject of investigations by Hartridge and Roughton, the solution of which may lead to certain modifications of the above conclusions. This problem has to do with the influence of the velocity of the reaction:

$$H_2CO_3 = H_2O + CO_2$$

upon the escape of carbon dioxide from the blood.

Nevertheless, the more general characteristics of our conclusions—their qualitative aspects and the orders of magnitude—appear to be well established as necessary consequences of laws that are known to be true.

It is safer, however, to take note that either a considerable increase of oxygen pressure in the lungs or a considerable decrease of oxygen concentration in active cells, accompanying increased metabolism, might lead to circulatory adjustments more complex than would otherwise take place. Under these conditions the blood might move with greater velocity through the capillaries. A similar state of affairs would exist if the diffusing process across the capillary wall could be accelerated in any other manner. But the blood in the muscles would still be little changed in composition.

The essential difficulty here, as in most truly physiological investigations, is but an expression of

that phenomenon of organisation, which is nowhere more clearly revealed than in the study of the respiratory function of the blood. In these lectures we have been led on, quite irresistibly, to the consideration of a steadily widening field, and of a constantly increasing variety of phenomena. To this tendency there can be no limit, except our natural incapacity to take account, simultaneously, of an indefinitely great number of variables. The strength of our natural powers of analysis is enormously reinforced by the nomographic method, which is, perhaps, destined to find in biological science its greatest usefulness. Sooner or later, however, any instrument of analysis, however powerful, must break down in the face of the vast complications of living organisation.

For the present, nevertheless, there is no immediate prospect of difficulty of this kind, except on account of the necessity of possessing, simultaneously, information concerning a great variety of different phenomena in one and the same organism.

The most recent researches of Bock and Field at the Massachusetts General Hospital have again considerably extended our knowledge of the respiratory function of A.V.B. The results of these investigations have to do with changes in blood flow, coefficient of utilisation of oxygen ($[HbO_2]_A - [HbO_2]_V$), pulse and total ventilation, with changes in muscular activity. The results are summarised graphically in Fig. 21.

Here muscular activity, measured by oxygen con-

sumption, is plotted against the variables in question in a manner which calls for no explanation. The

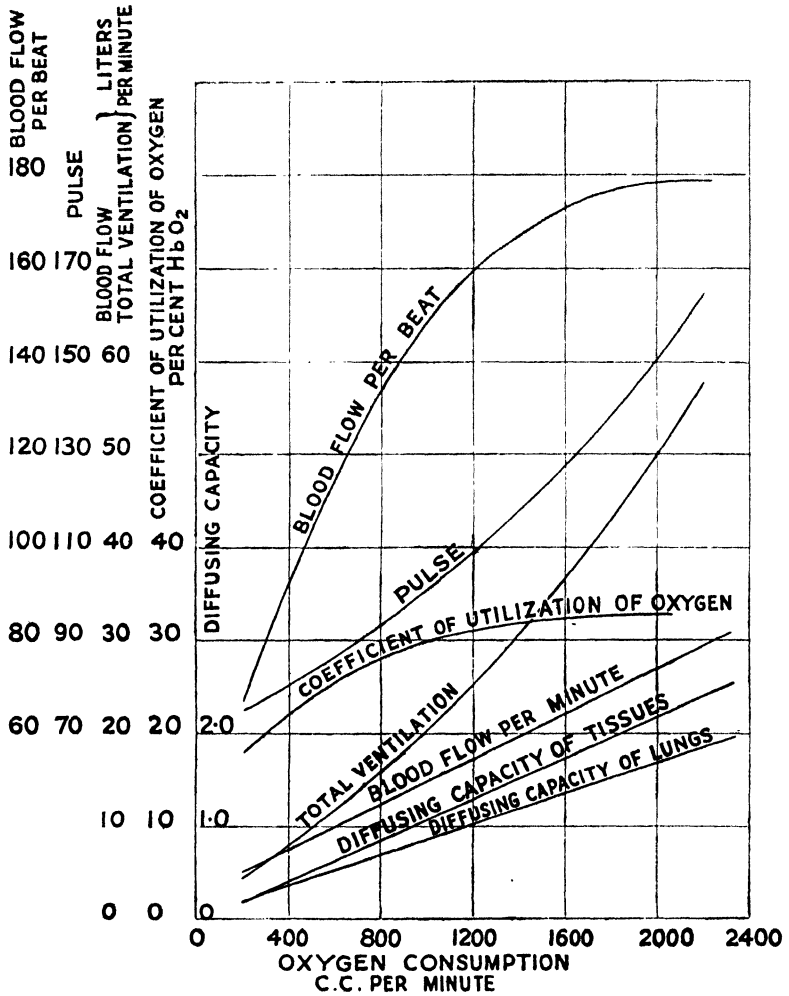


FIG. 21.—CIRCULATORY PHENOMENA IN A.V.B.

latest data are slightly different from those which have served for the earlier discussion of this lecture, but the difference is not significant for our present

purpose. For convenience rough estimates of diffusing capacity have been added.

It may be seen from a study of Fig. 21 that blood flow per heart beat and coefficient of utilisation of oxygen approach a limiting value, which may be assumed to represent the highest attainable efficiency, or at least a stationary efficiency. This is particularly striking in the case of the coefficient of utilisation of oxygen. Blood flow per minute is approximately a linear function of oxygen consumption. Pulse rate, and especially total ventilation, increase more and more rapidly with increasing activity, in a manner which suggests that they, or perhaps the latter exclusively, are the usual limiting factor. These results strongly support the conclusions which we have reached from the study of the diffusing capacity of the capillary bed.

A nomographic treatment of these more physiological aspects of our subject has been developed by C. D. Murray, and will be published in the *Journal of Biological Chemistry*. This investigation, and others which can now be undertaken on the basis of our present knowledge, may be expected still further to extend the synthetic description of the phenomena with which we have been concerned in this lecture.

* * * * *

At the end of this long discussion of the quantitative analysis of blood and circulation we may return for a moment to the philosophical opinions of Claude Bernard. The constancy of the alkalinity of blood

we have seen to be assured by certain unique properties of water and carbon dioxide, by the physiological control of the ventilation of the lung, by the admirable combination of properties of the hæmoglobin molecule, by the whole intricate heterogeneous equilibrium between red corpuscles and plasma, and, lastly, by the carefully regulated opening and closing of capillaries. In this respect Claude Bernard's hypothesis has received a confirmation more complete and more extraordinary than anything that he could have anticipated. The phenomena of water and chloride equilibrium, which are fully described by the large nomogram, but which we have not studied in detail, are only less striking confirmations of his hypothesis of the constancy of the internal environment.

Finally, it may now be seen that these three lectures are no more than an attempt to describe an integrated physiological phenomenon, the parts of which operate harmoniously and with a singular perfection to meet the changing conditions of the organism and of the environment. The first condition of life is survival, as Darwin perceived. Such integrated regulatory and compensatory activity as we have studied is, we may now see, the very process of survival in operation. Integration, harmony, constancy, are but necessary attributes of this process. Here, again, the opinions of Claude Bernard receive a clearer illustration. It is well to emphasise them upon occasion, for no physiologist may forget them with impunity.

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Two Lectures

By A. V. Hill

TITLE OF LECTURES

LECTURES

I. The Physical Environment of the Living Cell

II. Lactic Acid as the Keystone of Muscular Activity

LECTURE I

THE PHYSICAL ENVIRONMENT OF THE LIVING CELL

It is a fortunate thing for physiology that several of the generalisations of science appear to be strictly true even when applied to that complicated unit, the living cell. Ultimately the behaviour and characteristics of a cell must depend upon the chemical reactions which take place in the fluid media which surround it and make up its own structure. Rightly, therefore, we may turn to biochemistry as the ultimate means by which an analysis of that behaviour must be obtained. There is little use in trying to define a boundary between physics and chemistry, but in the usual sense of the words the chemical reactions which constitute, not merely the outward and visible activities, but the very essential nature of the cell, are dependent upon certain physical characteristics of its environment, as they refer to a unit of mechanism existing in a space incomparably smaller than any other mechanism—except the atom and the molecule—with which commonly science has to deal. It is with some of these physical characteristics, especially in relation to the minuteness of the engines upon which and with which they react, that I wish to deal to-day.

Although such exact experiments are not possible

on man, or on animals and plants, as may be made on non-living objects, there is no evidence that such living creatures do, or can, in respect of their outward behaviour, in any manner or degree, evade the ordinary laws of mechanics, of chemistry and physics, such principles as those of the conservation of energy and mass. At intervals, of course, especially in the public press, we hear stories of table-turning, of ghosts, of spirit photographs, of so-called electronic reactions for diagnosing disease, of medicines which can cure the evils to which our flesh is heir, in concentrations equal to that of a single ultimate molecule dissolved in a space equal to one hundred of our visible universes. If one is liberal-minded, as it is expedient for all scientific men to be, one occasionally examines the evidence—a disheartening process, and one subversive of liberalism. It is usually of the kind which requires a medium who is too shy to answer criticism, or a stream of special cases, or a faculty for explaining away the examples which do not confirm the theory. There is *no* evidence that momentum and kinetic energy, that chemical transformations, that surface forces, that electrical and magnetic phenomena, occur in the living body in any manner, or degree, which differs from that obtaining in the more readily investigated non-living world. The true biologist does not—he cannot—suppose that the phenomena of life can be explained in terms of present-day physics and chemistry; those sciences are still only skimming the surface of the world of natural phenomena. New theories will

be found necessary, new limitations, new corrections, new adaptations of old theories ; those endowed with a preference for the miraculous will, for a long time yet, be at liberty to assert that the generalisations of physics and chemistry have not been proved to apply to the material manifestations of the living creature. We can only answer that they have not been proved *not* to apply, and that it is our duty, our calling, to apply them. We can but continue to face the future with hope, mingled with a just scepticism.

If we study the exchanges of matter, or of energy, in living creatures, or in cells, we find, not perhaps with the same precision as in physics and chemistry, but with cumulative evidence, that these exchanges rest on precisely the same fundamental rules as they do in other sciences. Few would suppose that the laws of mechanics, the principles of the conservation of energy and mass, the atomic and the molecular theories, and the laws of electricity and magnetism, do not apply to the living cell in identically the same way, and with only the same ultimate limitations, as are found in the inorganic world. In dealing, however, with a mechanism so small as that of the cell we must beware of a loose and careless application of these laws. The theory of dimensions, when applied either to the very small or to the very large, leads us to conclusions which are often so startling as to appear to be the reverse of common sense. In biology physical common sense must be tempered by a due regard for the smallness and

adaptability of the cell, by an appreciation of the changes which come over our well-known physical laws when they are applied to structures of microscopic, or ultramicroscopic, size. It is conceivable, for example, that the second law of thermodynamics, dealing with the limitations of the availability of energy, may not apply, in an unmitigated form and under every circumstance, to the activities of the cell. It is known to rest on a statistical basis, and when we are dealing with units, complete and self-reproducing, yet as invisible and intangible as some micro-organisms can be, it is, theoretically speaking at least, possible that some means may be available of evading the statistical rules which govern the behaviour of larger systems.[1] I say only that this is *possible*; there is no evidence, as yet, of any definite character to suggest that the living cell can in any degree evade the jurisdiction of the second law. In a mechanism, however, in which probably chemical reactions may occur by the passage of one molecule at a time through the chemical machinery, it would seem but reasonable to attach more weight to the rules which govern single molecules than to those which apply only statistically to large numbers of them. Physiologists must accept no formulæ; they must attempt to understand the actual happenings.

The living cell is not so much a thing as a process; a chain of chemical events organised in a peculiar way. That organisation, that adaptation to the

[1] See Guye, *Physico-chemical Evolution*, Methuen, London, 1925.

needs of the animal of which the cell may be a part, is a thing which at present we can seldom analyse and understand ; it is rather a circumstance which we may but observe and admire. After we have completely analysed and understood all the individual chemical events and all the physical circumstances affecting them, we shall still be left with the much larger problem of how those events are organised to make up that collection of phenomena which we rightly recognise as an individual. It may at no very distant date be possible to describe all the events which occur, all the mechanisms which exist, in the muscle cell, where chemical energy is transformed into mechanical work, according to the needs of the animal. We shall still have to ask how that muscle cell, in its organisation and development, has adapted itself to the requirements of its owner, even to such things as his dimensions and the apparent value of gravity. Although the muscle cell of a tortoise and that of a frog may appear precisely similar, the one reacts to a stimulus twenty times as fast as the other. If the muscle fibres of an elephant's legs could shorten with a speed comparable to that of those which work the wings of a wasp, the inertial stresses set up by them in their owner's limbs would be such that he would have to spend his whole time remembering never to move too quickly. We may admit the embryological fact that the cell of the electric organ of a torpedo is the same in origin as that of his muscles ; we may even assume that the electric change which is invariably found in an active

tissue element is in principle the same as that which, in the electric organ, has been organised in series with those of similar elements to produce an electromotive force as great as that of the electric power mains. The forces or influences, however, which cause it to develop so, which provided it with the requisite insulators, which arranged the cells in series, will require a great deal more of understanding than the mere sequence of events which may be seen or detected during activity. At present we can do little more than attempt to study and analyse the physical and chemical accompaniments of activity, and observe, record, and admire the organisation by which that activity is made possible.

In the design of instruments—as Sir Horace Darwin has often told me—it is often expedient to discuss with oneself how they would appear if they were turned the other way round, what they would look like if their dimensions were reduced. In the study of biochemistry and of the physics of the cell it is often wise to take a book of physical constants and to go through it carefully, seeing how various things would seem when applied to a machine about one-thousandth of a millimetre across. One may spend many a happy and exciting hour with books of physical constants, and it is a pleasant and profitable amusement to go through their pages to see what startling conclusions one can reach. Take even the simplest thing: page 1, the c.g.s. units; has everyone realised that a cube 1 μ in height, of living substance, weighs only one-thousand-millionth of a

milligram? that the area of a 1 cm. cube when broken up into a billion 1 μ cubes is as large as 6 square metres? Even simple arithmetic can lead to some quite startling and amusing propositions. I hope you will forgive me, therefore, if I spend a few minutes straying still further in your company into this field.

A nerve trunk may be stimulated by a very small amount of energy; the propagated disturbance which we call the nervous impulse may be started by an electric current of such short duration and of such low intensity that its total energy is about $\frac{1}{1000}$ of an erg. Presumably the animal can stimulate his own nerves when he carries out his movements with an expenditure of energy no greater, probably much less, than that. Let us think what this means. One cubic centimetre of oxygen used in the combustion of carbohydrate or of any of the usual foodstuffs leads to a liberation of energy of about 5 calories; about two hundred million ergs; about two hundred thousand million times as much as is necessary to excite a nerve trunk once. Let us assume that a frog sends 200 impulses per second along a nerve to maintain the corresponding muscle in a state of voluntary contraction. If used entirely in providing energy to start these impulses, 1 c.c. of oxygen would serve him continuously for about one thousand million seconds, about 10,000 days, about thirty years. We may talk about mental energy: we may think we get very hungry when we use our brains; maybe we do: but the calculation shows that no great pro-

portion of the energy we use is required for setting impulses running in our nerves.

I open my book of tables at the page headed "viscosity of liquids": there one finds for the capillary tube method of determining viscosities Poiseuille's formula: viscosity $= \pi p r^4 t / 8 l v$: where p is the pressure difference between the two ends of the tube, r the radius of the tube, l its length, v the volume of liquid delivered in a time t. If you have a fancy for big numbers you immediately "spot" the factor r^4. Let us imagine a change from $r = 1$ cm. to $r = 0{\cdot}1\ \mu$, not so very small a radius, six times that of some of Einthoven's quartz fibres which he uses for recording sound, a size no smaller than that of many of the holes through which fluid must have to pass in the living cell. For a given head of pressure the volume of liquid delivered in time t will be diminished 10^{20} times: where we dealt with cubic centimetres we must now deal with molecules. Even if we imagine that our original 1 cm. tube has been broken up into ten thousand million little tubes so as to have the same total area, we still find that the volume of liquid delivered in time t is reduced 10^{10} times. Even if we further imagine that their length l is diminished in the same ratio as their radius the volume delivered will be only $\frac{1}{100,000}$ as large as previously. Clearly when we come to small, really small, dimensions viscosity will adopt quite a different rôle from that which it plays under ordinary circumstances. Such factors appear vividly enough in the dynamics of the circulation in the larger

animals, in the fall of pressure along the ultimate blood capillaries. They must play a still more vigorous rôle in the passage under pressure of a viscous fluid through the minute pores, or between the minute structural elements, of living cells. The cilia of a ciliated cell are of this order of size. The dynamics of the cilium must be largely affected by the viscosity of the fluid in which it works.

On p. 33 of one's book of tables one finds the number of molecules in a gram-molecule, 6×10^{23}, about 3×10^{19} to a c.c. We calculated before that a nerve impulse can be started by the energy obtained from the combustion of foodstuffs with one-two-hundred-thousand-millionth of a c.c. of oxygen. This represents about one hundred and fifty million molecules. If one hundred and fifty nerve cells can start the necessary process, each is allowed one million molecules of oxygen to provide it with the requisite energy: quite a respectable number after all!

Again, as we shall see in my second lecture, a muscle, when it develops a force of 1 dyne in 1 cm. of its length, liberates about 14 billionths of a gram of lactic acid. This contains some 9×10^{10} molecules. It is amusing, perhaps even instructive, to calculate the area which these would occupy if they were spread out in a continuous film such as that of a fatty acid upon the surface of water. According to the calculations and observations of N. K. Adam, such an acid molecule, resting with its terminal group pointing inwards to the water, occupies in a condensed film about 21 square Ångstrom units, that is,

21×10^{-16} sq. cms. The number of molecules, therefore, which a muscle liberates when, in 1 cm. of length, it develops a force of 1 dyne, would occupy an area of about one-four-thousand-five-hundredth part of a sq. cm. if arranged in such a condensed monomolecular film. If surface tension in such a layer is to cause the muscular response, the coefficient of surface tension must be four thousand five hundred dynes, clearly an impossible value. A little arithmetic, involving some rather large numbers, has in this case led us to a definite, possibly to a rather important conclusion; not less important, perhaps, because the area of the monomolecular film so calculated comes out to be about the same as the surface of the actual muscle fibres of which the muscle is composed.

Again, let us consider diffusion. To our ordinary senses, and in most respects, diffusion appears to be a very slow process. Apart from mixing and convection it requires days to equalise the concentrations, initially different, at two points a few centimetres apart. Let us assume that it takes eleven days, about a million seconds, for diffusion to equalise the concentration of a dissolved substance, e.g. in a jelly, at two points 1 metre apart. The laws of diffusion are such that, assuming for simplicity a state of linear diffusion, the equalisation takes place in a time varying directly as the square of the distance across which it occurs. Between two points, therefore, which are 1 μ apart, the time required is only one-billionth of that assumed for a distance of 1

metre: in this case, therefore, one-millionth of a second. Thus we see that diffusion, which works so sluggishly in the case of great dimensions, becomes almost fantastically rapid when we deal with small ones. Indeed, the size of living cells, the degree to which it is necessary for nature to break up living organisms into their ultimate units, depends largely, if not entirely, upon such considerations as the rapidity of diffusion. Diffusion in the living cell is the only, or almost the only, process by which chemical interchanges can occur between the interior and the environment. The size of the ultimate unit has been adjusted until diffusion plays a rôle no greater than that of other factors in the determination of the velocity of chemical interchange.

By a devious and perhaps unorthodox route we have arrived here at one of the most pertinent enquiries which biochemistry can make: that of the velocity at which, and the path by which, chemical change goes on. In ordinary chemistry many of the reactions are slow, some, without the aid of a catalyst, almost infinitely slow. Others apparently are instantaneous. Eliminating from our considerations such processes as those of an explosion, there are many reactions, the neutralisation of an acid by an alkali, the combination of ions with one another, the change of colour of an indicator, which seem to be so rapid that any attempt to study their velocity would be hopeless. A rapid chemical reaction is one whose speed is great compared with that of the other factors involved in bringing the reacting bodies into con-

tiguity. Mixing and diffusion on the large scale are usually so slow that any lag in the reaction as we observe it can be debited to them. In the living cell, however, the dimensions are so small that reacting bodies can be brought into one another's presence with such rapidity that the actual velocity of chemical combination must, in many cases, be the slowest link in the chain of processes. When we are dealing with the reactions of organic chemistry, in test-tubes, and observed by ordinary chemical methods, we are dealing with multitudes of molecules with little means available of isolating any of the intermediate products of the reactions; we are dealing in homogeneous media with an infinity of reacting particles. Thus we obtain products which represent merely the most probable result of an infinity of encounters. The most fundamental of all the problems of organic chemistry is that of the actual mechanism of its reactions: the path by which change takes place, the nature of the intermediate compounds. It is well known that in the living cell reactions may lead to different results from those produced by ordinary chemical methods. This is due presumably to the fundamental difference between the physical characteristics of the media involved. In the living cell the reactions may seem to be fast to the unsophisticated observer, but compared with the extreme rapidity with which diffusion can go on, with which the reacting bodies can be brought together or removed from one another's presence, they must be regarded probably as being in many

instances exceedingly slow. If there were any physical means of removing an intermediate product, for example a greater diffusion constant, a greater solubility, then intermediate products might be torn from the caldron of chemical change and dealt with along quite different paths from those which are decided simply by the laws of chance in homogeneous mixtures. Given a heterogeneous medium, with diffusion working a million times as fast as ordinarily, it may well be possible for the living cell to produce quite different results from the organic chemist. No doubt synthesis and breakdown by the living cell depend upon other factors, upon molecular "activation," upon surface forces, upon catalytic agents, upon "organisation" of many kinds. There comes, however, continually to one's mind the prospect that the very minuteness of its structure may be a determining factor in allowing it to deal so effectively, and apparently so arbitrarily, with its chemical processes.

If, therefore, on the time scale of the living cell, many of its chemical reactions be relatively slow, even those which to our sluggish senses seem almost infinitely rapid, then it is desirable, so far as possible, to study the actual velocity of chemical reaction by any means available. The contraction of a muscle is associated with the production of lactic acid. The sudden formation of that acid is rendered possible by the temporary "breakdown" of a barrier which usually holds the reacting substances apart. That barrier is probably molecular in dimensions, for

example a " passive " surface film. Such a breakdown constitutes that propagated disturbance which, in nerve, we call the nervous impulse. The acid produces its effect possibly by reacting with a protein existing in the muscle as a sodium-protein salt ; possibly by reacting with a monomolecular lipoid film. Such a process would be an ionic one. Similarly the relaxation of a muscle probably depends upon the neutralisation of the acid previously liberated : also an ionic change. The speeds of contraction and relaxation vary enormously from one creature to another, from one muscle to another in the same creature. The velocities, therefore, at which these chemical reactions occur must also be widely different. It would obviously be of the greatest interest to study the actual rate at which proteins and acids combine with one another, the actual speed at which the electric charge on an ionised colloidal surface can be neutralised. The older methods of chemistry have enabled one to follow very few reactions ; but recent work by Hartridge and Roughton at Cambridge has provided a method, of very general application, by which a number even of very rapid chemical reactions can be studied and their velocities estimated under various circumstances. In their work they have examined chemical changes which are complete in a few thousandths of a second, and obtained results which, but for the beauty and precision of their methods and their reasoning, it would be difficult to believe. The further development of their work may be of the greatest value in

elucidating the mechanism of the cell as a dynamic system.

The most striking and fundamental of the characteristics of a living cell is its power of reacting to a stimulus, that is to a change occurring often at a distant point. All living cells, some to a greater degree than others, possess the power of transmitting an influence of some kind which causes reaction at a distance. This transmitted wave-like influence is *not* the movement of material: the nerve fibre can transmit it with a speed of 120 metres per second, more than one-third of the velocity of sound: as W. B. Hardy said, it would take geological time for material change to occur, as the result of diffusion, along the nerve of a whale. The influence in question appears to be a self-propagating electrochemical disturbance, involving quite an incredibly small amount of energy exchange. So long as it is left unstimulated the nerve continues to exist quietly, without apparent change, in its environment, using only a very small quantity of oxygen to maintain its normal state. Stimulate it and a wave passes along it with only one known accompaniment, an electric action current, which is probably the agent by which one part "stimulates" the next and so causes the change to be propagated. Deprive the nerve of oxygen, or subject it to the action of a narcotising agent (which is fundamentally the same thing, according to Warburg's view, as the prohibition of oxidation), and in a short time, shorter if it be stimulated, it will fail to conduct its propagated influence.

Restore the oxygen, or remove the narcotic, and its previous power of transmitting a disturbance returns. The total amount of energy involved in the passage of such an impulse is immeasurably small. There must be some rise of temperature in nerve when the disturbance travels by, but this is certainly less than a hundred-millionth of a degree. The energy of the action current, which is the only physical phenomenon we can detect in an active nerve, is very much smaller than this. It is impossible, therefore, to regard the main body of the nerve as being the site of any considerable chemical change. It would seem far more likely that the change, whatever it be, occurs only in an infinitesimal part of the nervous material, and we are naturally led to look for it at one of the many surfaces which permeate a living cell. In the case of a nerve fibre we have a long, thin cylinder obviously separated by some impermeable or semi-permeable membrane from the surrounding fluid. If we suppose this surface membrane to be the site of the reaction which determines the propagated impulse, and if we suppose that the reacting film is only one or a few molecules thick, then we can understand why the amount of energy involved is so small. A monomolecular film of fatty acid deposited on the surface of a nerve fibre 10μ in diameter would occupy only $\frac{1}{5000}$ of its volume. Apparently, therefore, we must regard the active element in a nerve fibre as being a very thin, possibly a monomolecular, film of passive substance deposited and maintained on the nerve under the influence of

the oxidative reactions which go on slowly and normally during life. Hinder the oxidation and the film breaks down and the impulse can no longer be transmitted.

That such surface films, capable of transmitting an electro-chemical change, can exist in nature is well known, and in recent years Ralph Lillie in America has stressed the extraordinary likeness which exists in many ways between the change which is propagated in a nerve and that which can be transmitted in an iron or steel wire lying "passive" in nitric acid. An iron wire in nitric acid of specific gravity greater than 1·2 reacts only momentarily with the acid; a passive state rapidly sets in and for long periods of many days no kind of change appears to occur, either in the iron or the acid, in spite of the highly reactive nature of the substances which are, if not in contact, certainly within a few molecules' distance of one another. The iron develops a thin film, presumably of oxide, on its surface, which is maintained against chance small disturbances, no doubt, by a continual slow oxidation of the iron. Yet this seemingly stable system is in fact highly reactive: pass a local electric current through the iron wire, or touch it with a bit of zinc, and in a flash a change is propagated along the wire, it turns black, bubbles of gas come off, and in a few moments—longer in a dilute acid—it becomes passive once again. The iron wire transmits a self-propagating electro-chemical change, which in many particulars is analogous to the propagated impulse in a nerve. A physiologist has only

to see the demonstration to appreciate the analogy. Our more logical friends may laugh, perhaps, at our fondness for models; provided, however, that we regard them with due scepticism and reserve, models may be very useful; at any rate, they may emphasise the fact, which it is very desirable for a biologist to realise, that phenomena not unlike and not much less complicated or wonderful than those which occur in living substance, may also be observed in certain organised inorganic systems. Such analogies, provided that they do not make him think that he has solved his problem when he has made his model, may, at any rate, give him warning that "vital force" is not the only possible explanation of his observations.

Other cells than nerve cells possess the surface membranes or films which separate different chemical systems from one another. The red-blood corpuscle, for example, possesses in its envelope a membrane of highly specific properties on which depend very largely the properties of the blood. This membrane allows the free passage of oxygen, carbon dioxide, carbon monoxide, and other unionised substances. It permits the free passage of chlorine and hydrogen ions; it hinders absolutely that of sodium and potassium ions; movement across it is impossible to serum proteins and to hæmoglobin, so long as its surface is intact. Owing to its differential permeability it is doubtless subject to a complicated form of the condition first described by Donnan. That type of reversible thermodynamic equilibrium must undoubtedly exist at these interfaces, must un-

doubtedly produce differences of electrical potential across them. Probably it is one important factor in the surface phenomena with which we have to deal. It is certainly not, however, the only factor. In the blood corpuscle apparently the *status quo* can be maintained without continual oxidation in the cell. The red-blood corpuscle does not break down and lose its permeability or its characteristics if it be completely deprived of oxygen. In the red corpuscle we have presumably a simple case, one in which the purely thermodynamic equilibria play a preponderant rôle. In more active cells, however, the surface phenomena are probably bound up with continual slow oxidation, the oxygen being used in maintaining a reactive state by means of which changes may be initiated in response to stimuli acting at a distance. In the blood corpuscle, however, such reaction is not, or appears not to be, necessary. Although it may be the case that the blood corpuscle which we know, which we have experimented upon, is actually dead and that nobody yet has really studied a living red corpuscle, it would seem, if that contingency can be discounted, that in this particular cell we are dealing with a much simpler case to which the ordinary known laws of physical chemistry can be applied.

Recently at a meeting of the Physiological Society in Leiden, Gorter described some beautiful experiments which make it likely that the surface of the red-blood corpuscle is covered with a monomolecular film of some substance soluble in acetone. A number of red corpuscles was taken and their total surface

ascertained by measurement under a microscope and counting. The total acetone-soluble substance was then extracted from them and spread out upon a water surface in an apparatus of the Langmuir-Adam type. The area of the condensed film on the water came out regularly, in a variety of experiments upon a number of different types of blood, to be just twice that of the corpuscles from which the acetone-soluble substance had been extracted. This appears to suggest a film two molecules thick, a phenomenon rather difficult to understand. Dr. Garner, however, of this College, has suggested that if the substance in question had an acid group –COOH at each end, then at the water-water interface, of the red-blood corpuscle in contact with blood plasma, the two acid groups would be pointing in opposite directions and would occupy only one unit of the area. On the water-air interface, however, of the Langmuir-Adam apparatus, both acid groups would probably be pointing downwards into the water, so that each molecule would occupy two units of the area. The experiments of Gorter, therefore, are a striking indication of a line along which it may well be possible to explore more fully the nature of the surface films which seem so largely to determine the properties of living matter.

One thing may have struck you in this lecture: I have made hitherto little or no mention of the hydrogen-ion concentration. It would, indeed, have been a triumph to give a lecture upon this subject without any reference to a branch of physical

chemistry with which every biologist, however biological and however opposed in general to the extension of physical and chemical methods to biology, is conversant. To have done so, however, would be mere pedantry, and a triumph is costly at the expense of being reckoned a pedant. For, indeed, the hydrogen-ion concentration plays a quite extraordinary and preponderant part in determining the path and extent of the reactions which can be detected in organised living material. We are no more wrong now to stress the importance in biology of the hydrogen-ion concentration than our fathers were to emphasise that of the conservation of energy in the exact sciences. All life occurs in a watery medium, so far as we are aware, and in a watery medium it is natural to expect the hydrogen ion to play a fairly vigorous part. After all, looked at from the modern atomic theory, the hydrogen ion is nothing but a positive nucleus of electricity, the hydrogen atom deprived of its one electron, possibly delayed and hampered by the attentions of a number of dependent water molecules. Whether we should have expected it or not, the fact remains that the hydrogen-ion concentration plays a rôle of great intensity, that it affects every aspect of the living process. Oxidation and reduction are varied by its influence; the respiratory centre in the brain is excited by the smallest change of it; even the isolated muscle cell may have its activity reversibly abolished by blowing a little carbon dioxide through the salt solution in which it is bathed. The growth of bacteria, the

activity of ferments, the fertilisation of the egg, the activity of cilia, all depend, not to a small degree, but in a preponderating manner, upon the hydrogen-ion concentration. Much of its importance doubtless is concerned with the fact that living cells are largely made up of protein which under ordinary circumstances exists as an alkaline protein salt, the salt of a strong base with a very weak acid. Such salts are largely affected in their electrolytic relationships, they are ionised or unionised, by changes in acidity. The electrical discharge of a protein surface may liberate large forces, mechanical or physical, may set up tensions, may cause coagulation or dehydration, may produce extensive changes in the media in which cellular reactions occur. Possibly similar effects may result in the case of other substances, e.g. the so-called lipoid substances, as well as in that of proteins. In any case, simple observation shows the importance of this factor, and although much nonsense is talked and written about pH's, one cannot but admit the importance, both of accurate experimental work and—more especially—of accurate thinking on this somewhat complicated subject.

The progress of physiology depends to an extraordinary degree upon progress in other branches of science. The coming of the theory of electrolytic dissociation has wrought a wondrous change in the outlook of biologists. That theory, however, is still a very imperfect tool. We know that the parts of a salt break up into separate and oppositely charged bodies when in watery solution. Concerning the

force which prevails upon them to do so we know little or nothing. In a dilute solution of sodium chloride, how far apart are the sodium and chlorine ions? They are driven asunder by one force, held together by another: the latter presumably of an electrostatic nature, so that its magnitude can be calculated once we know the distance. To a biologist the actual nature of these forces is of paramount importance. In studying the cell we have certainly to deal with structures, more or less rigid, ionised at their surfaces, surrounded by a cloud of positive ions associated with the elements of negative charge on the surface. Such a system forms an electrical condenser possessed of so much electrical energy, subject to electric forces, parallel to the surface, at right angles to the surface. The formation of acid, so intimately linked with the mechanical response of muscle, may be the agent by which the electrical forces are released. Before, however, we can begin to calculate the magnitude of these forces and their characteristics we need to know the actual distance which separates the positive cloud of ions from the negative surface with which they are associated. Before, therefore, we can hope to make much progress we are dependent upon the work of physical chemists, who are at present debating this difficult and involved problem. Continually, in dealing with the electric manifestations of vital activity, we are brought up sharply by our ignorance of the ultimate nature of an electrolytic solution. Differences of potential may occur across a membrane: are they of the nature

of the contact potential which exists when any two material substances are caused to touch one another? Are they electrode potentials similar to that of a metal in contact with a solution? Are they of the type associated with the Donnan equilibrium? Are they due to oxidation and reduction? All these are problems which only further progress in physical chemistry can help us to solve. For the present physiology cannot be to any great extent a theoretical science; it must remain an observational and experimental one. For we may be quite sure that the theories which we shall require to explain our facts will involve physical conceptions totally different, much wider and more complete, than any which our colleagues in the other sciences have yet been able to provide us with. Theorising is good, theorising is valuable, and no man of science of intelligence and character will be deterred from building himself pictures of the way in which things work. For the present, however, until physics and physical chemistry can provide us with a much more complete picture of what actually happens in a solution than they can at present, we shall be wise to regard the pictures which we make for ourselves with a very liberal share of scepticism.

Another direction in which our science waits for a further development of knowledge in neighbouring sciences, is that in which we come upon the amazingly specific relations which exist between living cells and the various chemical substances with which they react. In some ways the chemical structure of the

living cell is analogous to the geometrical structure of a lock of almost infinite complexity. Substances similar in all their physical, and in nearly all their chemical, relations may show entirely different properties in their behaviour with living cells; and although the study of the action of drugs is one of the oldest of all the branches of science, we still have practically no clue as to the manner in which drugs exert their amazingly specific properties. Curare has long been said to hinder conduction of the nervous impulse at the end plate which lies between a nerve and its muscle. According to Lapicque and his co-workers, we should rather regard it as altering the "chronaxie" of the muscle fibre, a number which represents the "time factor" in stimulation. But why should it do that? Why should it not simultaneously alter the chronaxie of the nerve fibre? Why is it reserved for strychnine to do the latter? To take another simple case with which every medical student is conversant: why does veratrine cause a prolonged contraction of the voluntary muscle in response to a single shock? We have gone a short way in the answer to this question, but only to land ourselves in further perplexities. We know now that veratrine somehow modifies the surface layer, which normally holds apart the reacting substances which generate contraction, in such a way that when an instantaneous stimulus causes a wave of altered permeability to run along the tissue, there is not an immediate return to the passive condition after the wave has passed. In terms of our iron-wire model,

veratrine has somehow influenced the surface layer in a way which is analogous to what happens when the passive iron is placed, not in strong, but in dilute, nitric acid. So long as the wire is undisturbed no reaction occurs, but scratch it or pass an electric current through it, and the usual electro-chemical change is propagated, this time, however, without any return to the previous passive condition. The iron is now thrown into permanent contact with the nitric acid, and a reaction continues to occur which terminates only with the complete solution of the wire. If now our surface layer in the muscle, which normally holds the reacting substances apart, be really maintained by a continual process of slow oxidation going on in the cell, then how are we to regard a drug like veratrine as modifying its properties in such a curious way? We clearly need to know much more about the actual nature of these passive surface films, much more about their dependence on oxidation, much more about the return to passivity rapidly following a wave of excitation. We need to know the actual chemical structure, the geometrical structure, of the substances which make up this membrane, be they lipoid substances, or proteins, or something even more complex. We need to know the organic chemistry, the structural chemistry of the drugs, we require a geometrical picture to enable us to see how the lock is fitted by the key.

One single deduction I would make, if you will allow me, a conclusion drawn from my own experi-

ence. I was once a mathematician—indeed I took the Mathematical Tripos at Cambridge. My fellow-physiologists have always laughed at me about it, just as though I had once taken a degree in theology or in Sanskrit. But I have forgotten practically all the mathematics which I ever knew ; that, however, does not matter ; it seems to me that I can still think of things in a mathematical way, and I still find that the manner of thought which I learnt now more than half my life ago is of value to me in many things which I do. As it is with mathematics, so with physics and physical chemistry. We physiologists cannot hope to be adept in, to be really conversant with the development of, those other sciences. We should, however, have learnt them once and—if needs be—have forgotten them again ; they will linger unnoticed in our heads, unconsciously they will influence our actions, they will tell us the sort of problems which can be tackled by a physical or chemical method, they will give us a physical sense of proportion, they will tell us what is physically reasonable. And above all, they will give us, even though we have forgotten them completely in our conscious minds, a sufficient assurance to enable us to go over to the laboratories of physics or of physical chemistry, there to explain our difficulties in language which our colleagues will understand, and so secure that help which is always so generously provided by those colleagues, once they have realised our pathetic ignorance in the face of such appalling problems.

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LECTURE II

LACTIC ACID AS THE KEYSTONE OF MUSCULAR ACTIVITY

DURING the last twenty years very striking advance has been made in our knowledge of the intimate workings of the muscle, and, as is usual in science, precise knowledge gained in one direction has had important and interesting applications in others. Much more progress in the physiology and chemistry of respiration and of carbohydrate metabolism, even in the biochemistry of cancer tissue, has resulted from those old and persistent investigations of muscular fatigue which culminated in the classical work by Fletcher and Hopkins on lactic acid in muscle. The nature of muscular fatigue, the effects of which not only are visible in the intact animal, but are easily reproducible in the isolated muscle, has for many years attracted the attention of physiologists, and it has long been known that a fatigued muscle becomes acid owing to the liberation of relatively large amounts of lactic acid, $CH_3 \cdot CHOH \cdot COOH$, within it. The study of muscular fatigue, and of the conditions which affect it, is the chief cause which has led in the last twenty years to such revolutionary advance in the knowledge of the working of at any rate one type of living cell.

When a muscle, either in the body or isolated from it, is caused to carry out a regular and frequent series of contractions, a state of fatigue ensues in which the magnitude of the response diminishes, while the duration of its relaxation increases. Finally a state is reached in which the muscle is capable of no further response to a stimulus. The degree and rapidity of onset of the fatigue are largely affected by the circulation of blood through the muscle concerned. If two corresponding muscles in the two legs of an animal be subjected simultaneously to the same series of stimuli, the blood, however, being cut off from one of them, then the muscle with its circulation intact maintains its condition much longer than the other; the one without the circulation soon reaches a state in which no stimulus, however strong, can evoke any further response and which no recovery is possible so long as the circulation remains cut off. The muscle still possessing a circulation shows an initial diminution in the height of its contraction, but soon reaches a steady state at which it is able to continue contracting over long periods. In this state, recovery, as we should now say, balances breakdown. It might obviously be suggested that the maintained condition of the muscle still supplied with blood is due to the many materials, necessary for nutrition or required as fuel, which that blood brings it; that the supply of oxygen, however, is the important factor is shown by experiments in which two similar muscles, both deprived of their circulation, are subjected to the same series of stimuli.

the one muscle being in oxygen, the other in a gas free of oxygen. Provided that the muscle be sufficiently thin to allow a rapid enough diffusion of oxygen into its substance, it will, when stimulated in oxygen, soon reach a steady state beyond which—for long periods—no further fatigue occurs. The muscle without oxygen, however, soon becomes incapable of further response to stimulation, though—and this is a very important fact—it soon recovers its power if oxygen be admitted to it. Such observations on the importance of oxygen in maintaining, or in restoring, the capacity of a muscle for prolonged effort, suggested strongly that some substance was produced in muscular activity which accumulated in the absence of oxygen but did not accumulate, and might even be removed, in its presence. Since lactic acid was the only substance known to be liberated in muscular activity, and it is still—apart from inorganic phosphate—the only intermediate substance known with any certainty, it was natural to turn to a measurement of the lactic acid present in muscle under various conditions as the most hopeful method of analysing further the nature of muscular fatigue.

Even after seventeen years the methods available of isolating and measuring lactic acid are difficult and relatively inaccurate. In spite of this, however, the original paper by Fletcher and Hopkins in 1907 is still notable for the precision of its results and for the conclusive nature of the data and arguments presented. Fletcher and Hopkins found that when a

muscle is chopped or injured, or left in an irritating agent such as alcohol, there is a rapid production of acid. In heat rigor, or in that produced by chloroform, the production of acid is very rapid indeed. In a neutral non-irritating gas, such as hydrogen or nitrogen, there is a slow, steady production of acid at a constant rate, depending upon the temperature. The muscle cells, deprived of their usual means of obtaining by means of oxidation the energy necessary for their life process, have adopted what we may denote a "fermentative reaction" to obtain that energy. In air the production of acid is slower, in oxygen, even over considerable periods, it is entirely absent. In oxygen, provided that diffusion is rapid enough, the muscle cell can obtain all the energy it wants by oxidation. Diffusion is a very slow process, and the partial pressure of oxygen in air is not great enough to allow oxygen to diffuse sufficiently rapidly to inhibit the formation of acid. Consequently, in a muscle standing in air lactic acid accumulates, but not so quickly as in a muscle in nitrogen.

It is simple to demonstrate these facts indirectly in another way, namely by leaving two similar muscles, one in air and the other in oxygen, connected to levers to record their length. The one in oxygen does not shorten; it tends, indeed, to lengthen, and it never passes into the state of contracture which leads to *rigor mortis*. The one in air, however, and *a fortiori* one in nitrogen, gradually shortens and passes into the condition of *rigor*.

It was found not only that injury or prolonged survival without oxygen, but activity induced by stimulation, leads to the accumulation of acid to a concentration of 0·25 or more per cent. In a muscle so fatigued the lactic acid can be made to disappear by leaving it for a long time in oxygen. No disappearance, but a further increase occurs if it be kept without oxygen. Moreover, this process, in which lactic acid disappears after stimulation, was associated with a return to its previous contractile condition and to a disappearance of its contracture. Clearly oxygen carried out, or caused to be carried out, a "recovery process" in which lactic acid disappeared and the *status quo* was restored.

The process of inducing fatigue by stimulation, and then of allowing recovery in the presence of oxygen, could be repeated again and again any number of times on the same muscle. In isolated muscle treated in this way the recovery process was very slow, far slower than we should have expected from the known rapidity of recovery from muscular exercise in healthy normal individuals. In such individuals, however, the oxygen is brought immediately by the circulating blood into intimate contact with the muscle cells which require it. In the experiments, however, on isolated muscles oxygen is available only by the very slow process of diffusion. Observations on the rate of removal of lactic acid in isolated muscle give us no clue whatever as to the actual speed of the recovery process in the presence of sufficient oxygen. For this we must turn to

experiments on the heat-production of muscle, to which we will refer later.

One striking, suggestive, yet rather puzzling fact was brought out by the experiments on successive stimulation, fatigue, and recovery repeated a number of times: however much lactic acid had been produced and removed again, when the muscle was finally put into a state of rigor by warming it, the same amount of acid would appear as did originally in similar circumstances. An obvious explanation of this might be that the lactic acid was not really oxidised away, but simply restored to its previous position in some unknown precursor, the energy supplied by the oxygen used being employed in effecting the re-synthesis of this precursor. These observations raised acutely the question of what happens to the lactic acid when it is removed in the presence of oxygen. Subsequent work has shown that the reason for a constant maximum amount of lactic acid in rigor, or in stimulation, is not the limited amount of its precursor; the maximum is determined by a process finally inhibited by the products of its own reaction, in fact by the increased hydrogen-ion concentration caused by the formation of lactic acid: the presence of alkali around the muscle will allow the acid formation to proceed further. Later investigation, however, has entirely confirmed the suggestion that the lactic acid is not got rid of simply by being oxidised away, but by a re-synthesis to a precursor—glycogen. Indeed, as we shall hear later, the heat liberated in the recovery process is

adequate to account for the oxidation only of about one-fifth of the total amount of lactic acid which we know to disappear. It is this process of breakdown and re-synthesis of carbohydrate, to lactic acid and back again, which has proved so general a phenomenon in all kinds of living cells.

For further progress information was required which chemical methods, because of their relative crudeness, were incapable of supplying. If we wish to study the recovery process in its natural condition, it is necessary to ensure an immediate oxygen supply sufficient to account for all the oxidations required. Since an intact blood stream might supply or remove chemical bodies, to or from the active cells, and so complicate the interchanges to be observed, it was necessary to work with isolated muscles. In such, however, the diffusion of oxygen is so slow that the only oxygen available, for use in any reasonably rapid process, is that already present physically dissolved in the tissue fluids themselves. Oxygen is relatively insoluble, so that the amount of it immediately available, even when the tissues are in pure oxygen, is small. A pressure of several atmospheres of oxygen might ensure a greater available supply ; such an oxygen pressure, however, may produce abnormal results by injuring the cells. It is necessary, therefore, to work with breakdowns which are so small in extent that the whole of the oxygen present in the muscle in physical solution is not used up completely in the recovery processes resulting therefrom. The quantities to be observed, therefore,

are very small, and any method used must be very refined. Indeed the only possible one is that of measuring the heat-production.

There are many other advantages of measuring the heat liberated when a muscle shortens. It is possible to determine its time-course with accuracy, without injuring the muscle, continuously for as long as we please. The heat can be expressed in absolute units. It represents a very fundamental accompaniment and characteristic of the chemical reactions involved. Fortunately methods of measuring temperature by means of electrical instruments can be made so refined that there is practically no limit to the sensitivity available ; if necessary, it is possible to read to a millionth of a degree ; it is easy to obtain photographically the deflection of a galvanometer recording the rise of temperature of a muscle stimulated with one shock—a rise of temperature no greater than 0·003° C. ; and this rise of temperature can be expressed in absolute units, its time-course can be analysed, the heat liberated in the initial phases of contraction can be separated from that liberated in the recovery process. Naturally, when one is dealing with such small changes of temperature precautions must be observed, but given these precautions and a knowledge of electrical methods reliable results can be obtained. We must consider the results of myothermic experiments for the light they throw upon the actual course of the events accompanying—and following—a muscular contraction.

A muscle is placed upon a thermopile, in oxygen or in nitrogen, and stimulated. The galvanometer deflects, and its movements are recorded photographically. By suitable methods the deflection can be analysed, to give us a picture of the actual time-course of the production of heat. There are found to be four phases in the heat production. Firstly, at the moment when the muscle is stimulated, there is a large and sudden liberation of heat; then, as the stimulus is continued, heat continues to be liberated so long as the contraction is maintained; thirdly, heat is again liberated, while the muscle relaxes. These three phases constitute the *initial process* in muscular contraction, and a striking fact immediately emerges from the observations. Both the magnitude and the time-course of the production of heat in the initial phase are quite independent of whether oxygen be present or not. *The initial breakdowns in muscular activity are entirely of a non-oxidative character.* Now follows a recovery process. When the muscle relaxes the heat-production at first ceases. To outward appearances the muscle has returned absolutely to its former condition. If, however, it be in oxygen the heat-production now flares up again, its rate attains a maximum, and then falls back to zero, which it finally reaches in a period lasting for five minutes to half an hour, depending upon the temperature and on other circumstances. The total amount of heat given out in the recovery process is 1·5 times the total heat given out in the initial phases alone. The rate at which this heat

comes off—but not its amount—depends upon the hydrogen-ion concentration and upon the temperature, obviously also upon the condition of the muscle.

Let us see what conclusions can be drawn from these results. Firstly, it was natural to connect this slow evolution of heat during recovery with the removal of lactic acid known to occur: was the lactic acid oxidised? The heat measurements return an unequivocal answer to that question. It could be shown that in the initial process 296 calories of heat were liberated for every 1 gm. of lactic acid formed. If that acid were simply oxidised in recovery, it would liberate about 3,600 calories, about twelve times as much heat as was found in the initial phase. It was impossible that the observations of the recovery heat-production could be to that degree in error. The major portion of the lactic acid, therefore, had to be removed in some other way.

So matters stood when the war came and all things decent and sensible were forgotten. The next move made was subsequent to the war, this time again by a biochemist, by Prof. Otto Meyerhof, then in Kiel. Owing to Meyerhof's work it became possible to say, not only what was the origin of lactic acid when produced in muscle, but the general nature of its removal.

When a muscle is stimulated without oxygen, or kept in an oxygen-free state, lactic acid appears. Meyerhof showed that glycogen disappears in precisely equivalent amount. CO_2 is apparently formed. Actually it is driven out from pre-formed bicarbonate

by the lactic acid produced. Heat is liberated in proportion to the lactic acid formed, about 370 calories per gram.[1] If the muscle be stimulated isometrically the total tension developed in a long succession of contractions is proportional to the lactic acid liberated. The hydrogen-ion concentration rises a little, but so little that it is obvious that the lactic acid has been very completely neutralised by alkaline substances present in the muscle fluids. Meyerhof showed that the total amount of bicarbonate and phosphate present in the muscle is inadequate to account for the amount of lactic acid known to be neutralised. He showed, and subsequent experiments have amply confirmed, that the chief part of this neutralisation occurs at the expense of the buffered proteins of the tissues themselves. The muscle is really—both its solid and its liquid parts—a mixture of sodium protein salts possessing various isoelectric points, and when acid is liberated inside it sodium lactate is formed and the undissociated protein :

$\overset{+}{Na} + \overset{-}{P} + \overset{+}{H} + \overset{-}{L}$	$\rightarrow$	$\overset{+}{Na} + \overset{-}{L} + HP$
Sodium protein Lactic acid		Sodium lactate Undissociated protein

These results cleared the air. We had not to look for some mysterious lactic acid precursor ; we had only to deal with a straightforward—if complex—substance, glycogen, from which lactic acid was produced and then rapidly neutralised in the muscle

[1] This includes the " initial " anærobic heat, 296 calories, plus the " delayed " anærobic heat, 74 calories.

substance. That, however, was not the end of the story. It was still possible that reactions other than the formation of sodium or potassium lactate from alkali and glycogen might occur during muscular activity. The work of Meyerhof, however, followed by that of Slater, has shown that the total amount of energy known to be set free when 1 gm. of lactic acid is produced in muscle, is completely accounted for by the reactions we have described. If other chemical processes do actually occur they involve energy exchanges so small that we are unable to detect them. Hence we may conclude that the only important chemical reaction accompanying the initial stages of muscular contraction is that in which lactate is produced from glycogen.

The recovery process is a far more complicated matter. We still await further information as to its chemical nature. Much, however, is now known. In recovery lactic acid disappears, oxygen is used, carbon dioxide is produced. The extra CO_2 is equal to the extra oxygen. That is, the respiratory quotient of recovery is unity, carbohydrate, or something, e.g. lactic acid, of the same empirical formula, is oxidised. In the isolated frog's muscle there is no sign of any alteration in the fats, either in amount or in iodine value. In recovery, heat is liberated and (most important, perhaps, of Meyerhof's observations) glycogen is re-formed.

The amount of glycogen re-formed is not equal to the amount of lactic acid which has disappeared ; it is slightly less ; the difference is accounted for by

the oxygen used and the CO_2 produced; it may be calculated from either, or from the total heat set free, on the hypothesis that it is oxidised. The lactic acid which has disappeared is the precise equivalent of the glycogen which has reappeared, plus the amount of substance of general formula CH_2O which has been oxidised. The amount oxidised is about one-fifth of the quantity of lactic acid removed. The lactic acid is restored as the glycogen from which it came. One way of regarding the matter is to say that in order to supply the energy for this endothermic process one-fifth of the lactic acid has been oxidised to restore the remaining four-fifths. Another way is to say that the whole of the lactic acid has been re-formed, but that a small amount of glycogen, equivalent to one-fifth of the lactic acid, has been burnt to supply the energy for that endothermic reaction. It is impossible at present to decide which view is correct; only a knowledge of the actual chemical steps in these reactions can clear the problem up.

In the last few years this type of carbohydrate breakdown, the formation of lactic acid from sugar or glycogen in the absence of oxygen, followed by the restoration of the acid to glycogen afterwards at the expense of energy liberated in some subsidiary process, has been found to be a very general phenomenon. To take a simple case, Stephenson and Whetham have shown that *Bacillus Coli communis*, in an inorganic salt solution containing glucose, breaks down that glucose into lactic acid in the absence of

oxygen. In the presence of oxygen the lactic acid formation is diminished, oxygen is used, and CO_2 is given out. In a medium containing, not sugar, but ammonium lactate, in the absence of oxygen the bacillus was helpless, no growth or metabolism was possible. In the presence of oxygen, however, the lactate ions disappeared and CO_2 was formed. Far the most important development, however, of the subject has been due to Otto Warburg in Berlin. This is not the occasion to discuss Warburg's work, but it is clear from it that many types of tissue possess the same power of breaking down carbohydrate to lactic acid when deprived of their usual oxygen supply ; while considerable differences exist between different cells in their capacity for carrying on that biochemical sequence of reactions which, in the muscle cell, we call the recovery process. Fundamentally Warburg's work comes to this, that in cancer tissue the oxidative reactions which, in muscle, we associate with recovery have gone awry.

I am endeavouring in this lecture to show you that lactic acid is the keystone of the arch which now joins physiology, or, at any rate, the physiology of muscle, to the exact sciences. I will turn, therefore, to experiments of a very different nature, experiments on man undergoing violent exercise, which emphasise the same fact, the very fundamental nature of the place occupied by lactic acid in all forms of muscular movement. Lactic acid is known to occur in man ; it can be found in his urine after prolonged hard exercise. It is present in his blood at rest ; it

may be present there in very large amounts after severe exercise. Perhaps its most obvious sign, apart from direct chemical determination, is the respiratory distress accompanying and following hard effort. No other hypothesis than the formation of an acid, and its subsequent disappearance, could possibly explain the extraordinary changes of respiratory quotient which occur during and after work. In man it is possible to measure the amount of lactic acid set free during work by a determination of what, for lack of a better term, we have called the "oxygen debt." The oxygen debt at the end of exercise is the amount of oxygen required to restore the body to its previous resting condition. The magnitude of the oxygen debt is very large sometimes, and it can be shown that nearly the whole of the oxygen employed in recovery is used in effecting the oxidative removal of lactic acid formed during exertion. An oxygen debt of 1 litre is the equivalent of 7 gms. of lactic acid removed during recovery. It is comparatively simple to measure the oxygen debt. From it we may calculate the quantity of acid present in the body at any given time. In a vigorous man who has taken severe exercise the amount may be large, 100 gms. or more. Lactic acid is a relatively strong acid. The well-known subjective and objective symptoms of fatigue after severe exertion are due to its presence in the body.

The same type of recovery process occurs in man as in the isolated muscle. The lactic acid can be shown by direct analysis to disappear from his blood

in a period of about one hour following intense exertion ; after moderate exertion it disappears more quickly. In this connection, however, the indirect evidence is the more exact, and our knowledge of muscular activity in man has resulted chiefly from studies of the gaseous metabolism during and after exercise. By a collection, measurement, and analysis of the expired air of a man, it is possible with ease and accuracy to determine the oxygen used and the CO_2 eliminated. The respiratory quotient is the ratio of the CO_2 produced to the oxygen used. When the body is in a steady state the respiratory quotient is a sign of the foodstuffs being oxidised at the moment. In the case of violent muscular effort it is rather a sign of the formation and disappearance of lactic acid. During and immediately after a short bout of severe exercise, the respiratory quotient goes soaring up to values unheard of in the case of simple oxidation. It then returns towards its previous value, crossing it, however, and becoming very low while CO_2 is being retained by the body, to compensate for the previous loss following the sudden formation of lactic acid. During this period of low respiratory quotient the lactic acid is being removed and CO_2 is being retained to take its place.

The oxygen intake is a measure of the energy being used by the body. During exercise the oxygen intake is high ; when exercise ends it begins to fall ; it does not, however, fall at once to its previous resting value ; there is a considerable lag, many litres of oxygen being necessary after hard work

to restore the body to its previous condition. The recovery oxygen intake runs parallel to the changes observed in the lactic acid in the blood, and there can be no doubt that the recovery oxygen is associated with the oxidative removal of the lactic acid formed during exercise. It is possible, moreover, to determine, in the case of man, in two independent ways, the ratio of lactic acid removed to oxygen used in removing it. Again, in man, as in isolated muscle, we find that the lactic acid is not simply got rid of by oxidation, far more lactic acid is removed than can be accounted for by the oxygen used. The major portion of it is restored presumably to the glycogen from which it came, a small remainder only being oxidised in order to supply the energy for that endothermic reaction. The ratio of the lactic acid removed to the lactic acid oxidised comes out in man to have the same value as was found by Meyerhof, and independently by myself, by other methods in the case of the isolated muscle.

It can be shown, therefore, in man, that lactic acid plays just as preponderant a rôle in the chain of processes constituting muscular activity as it does in the isolated muscle. The importance of that fact is manifest ; its significance is that we may now make very many experiments on man which previously were never thought of because we had no established principle on which to work. In many respects man is an admirable subject for experiments, owing to the constancy with which the condition of his body may be maintained ; by reason of the control he is

able to exercise over it during an experiment, a man's body is often the most suitable experimental material for physiological observation. For the solution, however, of the really fundamental question we must return to the isolated tissue, where a greater variety of conditions and experiments is possible, where we can more freely isolate our variables and examine them one at a time.

By now I hope that all of you will have been asking yourselves: What is this lactic acid doing in all these strange changes through which it goes? Is it the actual agent by which the muscular mechanism is worked? Some people appear to have doubts about the answer to that question. Personally, I have very few. It has been shown that the formation of lactic acid from glycogen during the mechanical response of muscle is the only chemical reaction involving any serious energy exchange. If phosphoric acid be produced, as Embden indeed has demonstrated (and there is no doubt of the importance of phosphates in connection with carbohydrate metabolism), then neither does it cause any serious liberation of energy, nor does it result in any appreciable change of hydrogen-ion concentration. The heat produced is accounted for completely by the lactic acid formed, so probably is the change in hydrogen-ion concentration. P_2O_5, if liberated during muscular activity, is liberated as phosphate, at about the same hydrogen-ion concentration as the muscle fluids themselves, and not as phosphoric acid. Moreover, the mechanical response of muscle, as

measured by the tension developed in an isometric contraction, bears a fairly exact relation to the lactic acid set free. Lactic acid may not be a strong acid when compared with hydrochloric, but at the hydrogen-ion concentration existing in the muscle fluids it must be almost completely ionised. There, at any rate, it would be an extremely effective reagent. Personally, therefore, I have little doubt that lactic acid is the essential link in the mechanism.

Objection has been raised to this view by Bethe and others, because when a muscle is subjected to the action of an acid solution applied externally it does not shorten with the vigour associated with an ordinary contraction. Such an argument appears to me to rest upon a fallacy. No one imagines—so far as I know—that the acid produces its effect in causing the muscular response by a change which it evokes throughout the substance of the fibre: its effect must be highly localised at certain surfaces; as soon as it has left those surfaces it is neutralised in the general fluids of the cell. To assert that acid does not cause contraction, because acid poured on the outside of a muscle causes so much weaker a contraction than the natural one, is no more logical than to assert that the combustion of petrol is not the driving agency in a motor-car, because petrol poured over the outside of a motor-car and ignited will not cause it to go. There can, I think, be little question that whatever reactions produce the response of muscle those reactions are sharply localised in surfaces, either the surface of the muscle fibre itself, or possibly

that of the minute fibrils which are present in large numbers within it. To use Meyerhof's term, there is a *Verkürzungsort* where the acid acts, there is an *Ermüdungsort* where the acid is accommodated, presumably by neutralisation, after its action at the *Verkürzungsort*. A great variety of phenomena, far more than one can discuss in a single lecture, lead one to the view that the reactions determining mechanical activity occur at certain surfaces or interfaces in the fibre. The question, therefore, which I hope you are all asking yourselves is this: how, in point of fact, does this lactic acid produce its effect at some interface in the muscle fibre?

Unfortunately it is not possible to give a direct answer to this simple question. As yet the information available allows us to do little more than to make certain rather suggestive calculations and to propose certain rather indefinite hypotheses. I will start by giving you some data connecting the extent of the lactic acid production with the magnitude of the mechanical response which it accompanies, which we will suppose it causes.

In a muscle twitch, or in a very short tetanic contraction, the heat produced and the tension developed both vary with the length at which the contraction is carried out. When that length is the length of the resting unloaded muscle, both of these quantities are near their maximum, and for a certain range of lengths the ratio of tension to heat production is constant at its maximum value. For a given muscle

stimulated by a shock or a very short tetanus, at or near its unloaded length, the ratio $\frac{T}{H}$ is the same over a wide range of temperatures; this in spite of the considerable effect which temperature has upon other characteristics of the muscular response. There is every reason to suppose that H, the heat production, is a measure of the amount of lactic acid liberated, so that apparently the magnitude of the mechanical response is constant and independent of temperature for a given amount of lactic acid produced. If now we wish to compare the ratio $\frac{T}{H}$ in different muscles, either different muscles of the same animal or muscles of different animals, it is necessary to introduce some factor to take account of the dimensions of the individual muscles concerned. Since T is of the dimension of force and H is of the dimension of work, it is clearly necessary to multiply T by some length to make it of the same dimension as H. The obvious length to take is that of the resting unloaded muscle. There is an excellent physical reason for doing this: if a muscle be twice as long, the tension developed will be the same, but the heat production will be twice as great; if the muscle be twice as thick, both the tension and the heat will be doubled; thus no dimensions except the length need be taken into account. If now we study the quantity $\frac{Tl}{H}$ we find it to be very constant as we pass from one muscle to another in the same

animal, and even as we pass from one species to another, for example, from the frog to the tortoise. Expressing T in dynes, l in centimetres, and H in ergs, the maximum value of $\frac{Tl}{H}$ comes out uniformly at about 5; in other words, the quantity Tl bears to the amount of lactic acid produced in the twitch, a constant ratio in different animals, in different muscles of the same animal, in different species, and at different temperatures. Clearly in this quantity we have something which approximates to *a natural constant for muscle.*

Now we know the amount of heat liberated in the initial processes of contraction when 1 gm. of lactic acid is liberated: it is about 296 calories, about $1{\cdot}24 \times 10^{10}$ ergs. From this we may calculate the amount of acid set free when any given amount of heat is liberated in a muscle. Let us consider the formula $\frac{Tl}{H} = 5$. This implies that when a force of 1 dyne is developed in 1 cm. length of muscle, the heat which accompanies the contraction is equal to 0·2 erg, which is the equivalent of $16{\cdot}1 \times 10^{-12}$ gm. of lactic acid. Thus a muscle fibre 1 cm. long, developing a force of 1 dyne, must liberate simultaneously about 16 million-millionths of a gram of lactic acid.

Approximately the same result can be obtained from certain independent facts due to Meyerhof. Meyerhof carried out experiments on frog's muscles, in which he compared the total tension developed in

a long series of muscle twitches with the total lactic acid set free. He expressed his results in terms of what he called the isometric coefficient for lactic acid. His isometric coefficient he defined as follows :

$$K = \frac{\text{(total force developed in kgms.)} \times \text{(length in cms.)}}{\text{(mgms. of lactic acid produced)}}$$

Expressing everything in absolute c.g.s. units Meyerhof's results may be stated in the form: gms. of lactic acid produced $= \frac{Tl}{K} \times 1{\cdot}02 \times 10^{-9}$. For fresh semi-membranosus muscle Meyerhof found a value for K of 78 ; for unfatigued gastrocnemii one of 123. The difference between the two types is due to the fact that in the gastrocnemius the fibres do not run the whole length of the muscle, whereas in such muscles as the semi-membranosus or sartorius they practically do. Certain independent data show us that on the average it is necessary to assume that in the gastrocnemius the fibres run only 63 per cent of the length of the whole muscle. It is necessary, therefore, to multiply the isometric coefficient for gastrocnemius muscles by 0·63 before comparing it with that for the adductors. This gives 77·5, practically the same as the value for the other muscles. Applying the above formula, we then find, gms. of lactic acid $= Tl \times 13{\cdot}1 \times 10^{-12}$. Thus, accepting Meyerhof's data we find that a muscle fibre developing a force of 1 dyne in 1 cm. of its length, must liberate 13·1 million-millionths of a gram of lactic acid. The agreement of this value with that calculated

from independent data is striking testimony to the general validity of our argument. Let us take a mean value, therefore, namely, $14{\cdot}6 \times 10^{-12}$ gm. of lactic acid as the amount liberated when a fibre 1 cm. long develops a force of 1 dyne. In any quantitative discussion of muscular contraction we may start from this number as a basis.[1]

It has often been supposed that muscular contraction is due to a change of surface tension provoked by some substance liberated on stimulation. Certainly no substance is liberated in greater quantities than is lactic acid. It is instructive, therefore, to calculate the area which a film of lactic acid 1 molecule thick would occupy if spread out upon some surface in the muscle fibre. Calculated from its density the area occupied by 1 molecule of lactic acid should be about 24×10^{-16} sq. cm. According to N. K. Adam, the area occupied by a fatty acid molecule forming part of a condensed film on the surface of water is 21×10^{-16} sq. cm. Accepting Adam's value, the total area of $14{\cdot}6 \times 10^{-12}$ gm. of lactic acid, which is almost exactly 10^{11} molecules, if spread out in a monomolecular film, is 21×10^{-5} sq. cm.; not far from $\frac{1}{5000}$ of a sq. cm. Thus, when a muscle fibre 1 cm. long develops a force of 1 dyne, the area occupied by the lactic acid molecules

[1] It may be of interest to record that approximately the same number, viz. 23×10^{-12}, may be calculated roughly from certain observations communicated to me privately by Dr. A. C. Redfield, of Harvard University. These observations were made on the heart muscle of the tortoise.

liberated is about $\frac{1}{5000}$ of a sq. cm. Now the length of this area, on any hypothesis of surface action, we may assume to be 1 cm., i.e. the length of the muscle fibre under consideration. The width of the area, therefore, is $\frac{1}{5000}$ of a cm. The force of 1 dyne has to be produced on an edge $\frac{1}{5000}$ of a cm. wide; if that force be due to surface tension, the coefficient of surface tension must be 5000 dynes per cm. The surface tension of water is 73 dynes; that existing at a water-olive-oil interface is about 21 dynes. Clearly we have arrived at quite an impossible value. Muscular contraction cannot be due simply to a change in ordinary surface tension produced by the lactic acid liberated: there is not enough of the latter.

It might at first sight be supposed that lactic acid could affect the surface tension at an interface without being distributed in a complete monomolecular film. If we assume a change of surface tension to occur, equal to that which exists between olive-oil and water, the area occupied must be some 250 times as great as that calculated. It is impossible to imagine that an occasional lactic acid molecule, occupying only 0·4 per cent of a surface, could effect a change in surface tension as great as that existing between oil and water. Unless something else is produced in amounts enormously exceeding that of lactic acid, it is impossible to suppose that a change of surface tension is the determining agent in muscular contraction.

Many facts, however, do lead us to the view that

the reactions which underlie the response of muscle occur at surfaces or interfaces in the fibre. There are other forces besides that of surface tension which might act at such surfaces. In a recent letter to *Nature*, Garner suggested that many of the phenomena of muscular activity might be accounted for on the hypothesis of a film of liquid crystals, composed of some lipoid-like substance, deposited in the anisotropic portions of the fibres. The production of acid might cause a change in the angle between successive elements of this crystal lattice, or might otherwise alter its geometrical condition, and so bring relatively large forces into play without necessarily occupying the whole area with the molecules liberated. Or, again, we might imagine that the fibrils visible inside the fibre consist of protein substance negatively charged with a cloud of positive ions around them. Each cylinder of protein negatively charged, with its attendant cloud of positive ions, would in effect constitute an electrical condenser. Such a condenser would be in a state of strain under the mutual repulsion of the elements of charge occupying its plates. The sudden liberation of lactic acid in the neighbourhood of the negatively charged protein surface would cause a discharge of the condenser by the formation of sodium lactate and ionised protein. The mutual repulsion of the charges would then be obliterated and the condenser would tend to shorten. The force developed in such a condenser suddenly discharged can be calculated [1]

[1] For this calculation I am indebted to Prof. Ehrenfest of Leiden.

provided that we know its dimensions and the density of its charge. With certain possible assumptions the force developed so calculated, on the hypothesis of a monomolecular film of lactic acid deposited on the negative plate of the condenser, is quite large enough to explain the data at our disposal. A force of 5000 dynes per cm. of edge would seem to be perfectly possible as the result of such an electrical discharge. In principle the hypothesis rests upon the same phenomenon as does the capillary electrometer, an apparent rise of surface tension when a surface is discharged electrically.

The hypotheses suggested are still vague and their usefulness must be tested by future experiments; it may, however, be useful, before concluding, to give you a few short calculations of the area available in muscle at the interfaces visible under a microscope. A homogeneous frog's muscle, such as the sartorius, is composed of fibres averaging about 50 μ in diameter. A frog's sartorius weighing 150 mgms. and 3 cms. long can, under favourable circumstances, develop a force of about 150 gms. weight, about 150,000 dynes. Assuming the muscle to have the same specific gravity as water, calculation shows that its area of cross-section is 5 sq. mm. Per sq. mm., therefore, it can develop a maximum force of about 30,000 dynes. In this sq. mm. there must be about 400 fibres, each of 50 μ diameter. Each fibre, therefore, must be able to develop a force of about 75 dynes. Its circumference is 157 μ, or 0·0157 cm. Hence, per cm. of circumference the maximum force developed

by the fibre is $\frac{75}{0{\cdot}0157} = 4800$ dynes. It is striking that this value is the same as that calculated per cm. of edge on the hypothesis of a monomolecular film of lactic acid.

This last result can be put in another way. When a frog's muscle contracts it liberates so much lactic acid. In the maximal contraction of a muscle in good condition the amount of lactic acid set free, if spread out in a film 1 molecule thick, would be able to occupy an area approximately equal to that of the actual muscle fibres of which the muscle is composed. Whether this result is a coincidence or not one cannot say ; it certainly suggests that the surface of the muscle fibre itself may be the *Verkürzungsort*, the place at which the lactic acid works. The agreement, however, may be due to nothing but chance, and we must not be led astray by chance. There are other surfaces or interfaces in the muscle fibre, at any rate after it has been killed and fixed and stained.

Inside the individual muscle fibres, visible under the microscope, are the muscle fibrils, stated to be about 1 μ in diameter, about 31 million of them to the sq. cm. of muscle. In 1 cubic cm. of muscle it may be calculated that the area of all the fibrils which it contains is about 20,000 sq. cms. In our calculation above we have assumed that the muscle can exert a maximum force of 30,000 dynes per sq. mm. This is 3 million dynes per sq. cm. Thus, for every centimetre of edge, on the hypothesis that

the fibrils are the agents involved, the maximum force exerted by the muscle is 150 dynes, only about $\frac{1}{33}$ of that calculated previously. Thus, in the maximal contraction of a muscle the amount of lactic acid set free would be sufficient to cover, in a monomolecular film, only about $\frac{1}{33}$ of the area of the ultimate fibrils visible inside the fibre. If, therefore, some hypothesis similar to that of Garner's were correct, we might suppose that, in a maximal contraction, about $\frac{1}{33}$ of the area of the muscle fibrils is occupied by lactic acid molecules suddenly set free, with the consequent effect upon the disposition of the liquid crystals there situated.

There I must end. Some of my calculations may have seemed to you wild, my hypotheses improbable ; they are, however, but attempts to rationalise the quantitative results of recent work, and the latter are fairly accurate. These provide a basis on which we may treat the muscle as a machine possessing certain known physical characteristics. It is only when the properties of a substance, of a machine, of an effect, can be expressed in absolute units, that we may hope to treat the problem involved as a physical one.

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