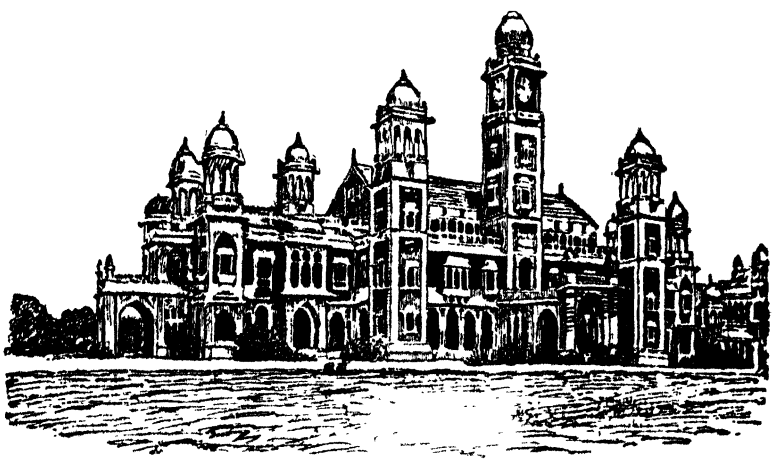
UNIVERSITY OF ALLAHABAD STUDIES

ZOOLOGY SECTION



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CYTOPLASMIC INCLUSIONS IN THE OOGENESIS OF SOME FOREST INSECT PARASITES *

BY

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* The work was carried out during the tenure of Empress Victoria Readership, 1937—40, at the University of Allahabad.

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PREFACE

The present paper (abridged) is Part II of the thesis accepted for the degree of Doctor of Philosophy, University of Allahabad, 1940. The first part dealing with the Entomological investigation on the parasites of pests of *Dalbergia sissoo* was carried out in the laboratories of the Forest Entomologist, Forest Research Institute, Dehra Dun, and Zoological laboratory, University of Allahabad; in the latter laboratory the cytological part, in addition to entomological, was carried out.

The research work was conducted under the able guidance of Dr. C. F. C. Beeson, C.I.E., D.Sc., I.F.S., Forest Research Institute, and Professor Rai Bahadur D. R. Bhattacharya, Ph.D., D Sc., F N.I., Professor of Zoology, University of Allahabad. I received valuable suggestions from Mr. J. C. M. Gardner, I.F.S., D.I C., Forest Entomologist, Forest Research Institute, Dehra Dun, during the course of investigation. To all, my grateful thanks are due.

It would have been impossible to complete the investigation without the facilities provided by the President, Forest Research Institute, Dehra Dun, to work in the laboratories of the Forest Entomologist, and I feel so grateful for the same. The award of the Empress Victoria Readership by the University of Allahabad is gratefully acknowledged.

I was associated with Mr. S N. Chatterjee, F.R.E.S., Forest Research Institute, throughout the investigation and received much valuable criticisms and ideas and am grateful for the same.

The paper was written and the major portion of the work was carried out in the Zoology Department, University of Allahabad, under the guidance of Professor Bhattacharya for which my sincere thanks are due.

INTRODUCTION

Practically no study has been done on the cytoplasmic inclusions in the oogenesis of forest insect parasites except the one recently, by Chatterjee (1938) Gatenby earlier studied the braconid *Apanteles glameratus*.

The material of the present investigation consisted of the following forest parasites :—

1. *Cedria paradoxa* Wlkn., a braconid parasite of teak skeletoniser, *Hapalia machaeralis* Walk.
2. *Trichomma nigricans* Cam., an ichneumonid parasite of teak skeletoniser, *Hapalia machaeralis* Walk.
3 *Dioctes gardneri* Cush., an ichneumonid parasite of teak defoliator, *Hyblaea puera* Cram
4 *Rhogas plecopterae* Chatterjee, a braconid parasite of shisham defoliator, *Plecoptera reflexa* Guen
5. †*Enicospilus* sp., an ichneumonid parasite of shisham defoliator, *Plecoptera reflexa* Guen

There have been technical difficulties in this work, the foremost being the paucity of female parasites, on account of which much of the experimental work was abandoned. Ovary being microscopic got lost before reaching to the stage of block making. A recourse was taken to fix the abdomen with the ovary intact. This involved imperfect penetration of the fluids and a problem in sectioning (paraffin blocks) arose because of the chitinised abdomen. Softening of the chitin would have rendered the study useless for purposes of the study of the

† The new species will probably be described by Cushman of the United States Museum.

cytoplasmic inclusions. Slightly thicker sections, 4 to 5μ were cut with Minot's Microtome, to avoid rupturing.

The modern technique as given in the recent edition of Vade Mecum (1937) has been employed in the present investigation. For deciding the origin of fat bodies the Mann-Kopsch preparations have been bleached with pure turpentine. The observations on the Golgi bodies are based on the Mann-Kopsch technique with a fortnight's osmication in the laboratory temperature. The observations on the mitochondria and the behaviour of nucleolus are based on the Chrome-osmium techniques; yolk bodies are also studied in these For the detection of fat, Sudan III, Scharlach R and Sudan IV were used.

Living ovariole were studied (a) unstained (b) stained with neutral red for the demonstration of 'vacuome' of Parat (c) stained with Janus green B for the demonstration of mitochondria, (d) stained with Sudan III and Scharlach R for the demonstration of fat bodies and (e) stained with 2% osmic acid for the demonstration of Golgi bodies

The stains Neutral Red and Janus Green B were freshly prepared according to Bhattacharya's formula as given in Vade Mecum.

For the stratification of the various cytoplasmic inclusions of the egg, material was centrifuged electrically at a speed of 3,250 revolutions per minute in 0 75% salt solution, for about four hours Then the material was fixed in Champy and stained with scid fuchsin and methyl green.

In the present work cold fixation was done throughout.

PERSONAL OBSERVATIONS

(a) With fresh material.

(1) *Unstained material.*

Ovaries of *Cedria paradoxa*, *Dioctes gardneri* and *Trichomma nigricans* have been studied in the living condition in the physiological salt solution. When the ovariole is examined under the high power of a microscope, each nurse chamber is found to contain several nurse cells, the nuclei of which are round and big with a thin strip of cytoplasm; the walls of the nurse cells in the Ichenumonidae appear to be polygonal and thus a complete picture of the nurse cells inside the nurse chamber appears to be that of a bee-hive In the later stages an optical look shows a direct continuity between the nurse and oocyte chamber with flowing cytoplasm from the former to the latter. Inside the mature eggs reserve food material is found in abundance, which gives them a dark appearance under the microscope Under the coverslip are observed numerous dancing organelles; the dancing appearance is exactly like the play of a child with a tennis ball against the ground, the movements being very quick. Some of these are optically vesicular with distinct boundry line the interior being dull yellowish. These are recognised as Golgi bodies which are revealed by the application of 2% osmic acid, as dealt with later on. Some of these dancing organelles (more numerous than the Golgi bodies) are optically granular, smaller and light bluish in colour. These are recognised as mitochondria revealed by the application of Janus Green B observations on which are dealt with later. These inclusions observed in the living ovariole have been found in all the three categories of cells, but in great abundance in the oocytes.

(2) *Neutral red staining.*

With the application of neutral red the entire ovariole becomes visible fairly well, probably due to its diffuse staining throughout. The application of this dye increased the visibility of Golgi bodies, which apparently seem to be tinged with the dye. The vesicular nature of these bodies also becomes marked. Simultaneously it was observed under the oil immersion lens that pink bodies of various sizes were appearing and their number increased; these pink spheres (*Parat's 'vacuome') were quite distinct in mature eggs as well as in nurse and follicle cells. It was also seen that at places tinged Golgi bodies and the 'vacuome' touched each other forming a figure of eight. In this very preparation 2% osmic acid was slowly trickled down under the coverslip. Immediately the duplex character of the Golgi bodies appeared with a distinct chromophilic rim and a chromophobic centre; crescents were also visible. Figures of eight were seen by the association of Golgi ring and 'vacuome'.

(3) *Janus green B staining.*

The ovarioles were stained in a very dilute solution of Janus green B (Fig. 24). The granular mitochondria appeared blue within 20 minutes; in the advanced eggs filamentous types also appeared but in the fixed preparations as will be seen later, only granular types were seen in the chrome-osmium fixatives except in *Rhogas plecopterae* where long filamentous mitochondria appeared in the mature eggs. In the advanced eggs the yolk bodies also took the stain of Janus green B. With prolonged application of the dye Golgi bodies with duplex character were visible.

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* Singh and Boyle (1938) remark " . . . it is sufficient to say that . . . by using ultracentrifuge . . . no 'vacuome' exists in animal cells . . . Parat's 'vacuome' . . . has been recently shown to be an artefact . . . ."

(4) *Sudan III and Scharlach R.*

The ovarioles of *Cedria paradoxa*, *Dioctes gardneri* and *Grichomma nigricans* were stained in the alcoholic solutions of Sudan III and Scharlach R for the detection of fat bodies. It was found that bright red bodies appeared within 45 minutes; they were found in advanced eggs and also in the earlier oocytes but the fat globules of the latter were smaller in size than those of older eggs; at some places very big and bright red bodies were seen in the older eggs. This test does not furnish any proof as to the origin of these fat bodies.

(5) *Sudan IV.*

The recent technique of the microchemical tests for fat with *Sudan IV has been tried with four species of forest parasites, namely *Euplectrus parvulus* Ferr., *Disophrys sissoo* Wlkn., *Microgaster plecopterae* Wlkn., and *Apanteles macheeralis* Wlkn. In the eulophid *Euplectrus parvulus* the sudanophil fat bodies did not show out distinctly but the latter bodies appeared as bright red and the haemalum stained the proteid yolk bodies blue in the braconids In the older eggs the sudanophil bright red fat bodies and the haemalum stained blue proteid yolk bodies were seen intermixed.

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* On account of the paucity of female specimens of *Cedria*, *Dioctes*, *Trichomma*, *Encospilus* and *Rhogas* the Sudan IV technique could not be tried at the time the chemical was available; therefore, other parasites were utilised to carry out the test with the new stain.

The staining in Sudan IV was done by fixing in the formalin (40%) for 10 minutes; washed quickly in 50% alcohol; in Sudan IV for 25 minutes; two quick washes in 70% alcohol; differentiation in haemalum for 5 minutes; quick wash in 70% alcohol and examination under glycerine mount. Sudan IV and filtered haemalum were prepared as given in Vade Mecum by Gatenby (1937).

(b) With fixed material.

NURSE CELL.

Golgi bodies.

Cedria paradoxa.—The Golgi bodies of the earliest nurse cells appear as vesicular bodies. They are mostly found in the perinuclear zone. Some of these vesicles with a thin chromophilic rim are found scattered in the general cytoplasm of the nurse chamber Later they are uniformly distributed.

Rhogas plecopterae —The Golgi bodies of the earliest nurse cells appear as a couple of elements sticking to the nuclear membrane which gradually increase in number and get concentrated for the most part in a perinuclear zone. At first they form a cap to the nuclear wall and then a perinuclear zone Finally the Golgi bodies are uniformly distributed The Golgi bodies are vesicular in shape.

Enicospilus sp.—The Golgi bodies of the earliest nurse cells as examined are small in size and some are vesicular in shape; these bodies occupy a juxtanuclear position in the older nurse cells The Golgi vesicles disperse after attaining the perinuclear stage (Fig. 15); they are very few in numbers. Intermixed with the vesicular bodies granular and crescentic forms are also present.

Dioctes gardneri.—The Golgi bodies of the nurse cells (Fig. 11) in the earliest stage are found in the perinuclear zone; later they disperse in the cytoplasm. The Golgi bodies are granular and vesicular with distinct chromophilic rim, according to the optical picture obtained under the oil immersion lens. These are comparatively of a bigger size than the other species and are distinctly seen in the Mann-Kopsch preparations

Trichomma nigricans.—The Mann-Kopsch preparation treated with turpentine for a very short period

showed that the Golgi bodies decolourised very quickly in the nurse and follicle cells which prior to bleaching were seen as black and vesicular bodies.

Mitochondria.

Cedria paradoxa.—In the youngest nurse cells the mitochondria appear in a fine granular form in the perinuclear zone and the latter greatly widens in the older cells (Fig. 3). Finally they are uniformly distributed. In the advanced stage it has been observed that the inclusions of the nurse cells pass down along with the cytoplasmic stream into the developing oocyte.

Rhogas plecopterae —In the youngest nurse cells the mitochondria appear as few granules; next they appear as a cap and then form a perinuclear zone; they are extremely fine and granular. Later they are uniformly distributed in the cytoplasm The developing oocyte is nourished by the nurse cells, the contents of the nurse cells pass into the growing oocyte along with the cytoplasmic current. The Golgi bodies also flow with the stream. A complete absorption of the nurse chamber by the oocyte has not been noticed here (Fig 5)

Enicospilus sp.—In the youngest nurse cells examined the mitochondria form a prominent dense perinuclear ring and the rest of the cytoplasm contains scattered bodies. They are granular throughout and this condition persists upto the late stage of oogenesis (Fig. 17). As observed with the other species of parasites the inclusions of both types flow from nurse cells into the growing oocyte along with the cytoplasmic stream (Figs 16, 17).

Dioctes gardneri.—The mitochondria of the nurse cells (Fig. 13) in the earliest stage have been found in the perinuclear zone. Later they get scattered in the cytopalsm. The mitochondria are granular throughout the course of oogenesis. In this species mitochondria have responded nicely to the chrome-

osmium technique. The inclusions of nurse cells infilter into the ooplasm in later stages. The Golgi bodies have also been observed to flow along the cytoplasmic stream from nurse cell to oocyte, thus throwing the inclusions of the oocyte to the periphery (Fig. 12).

Trichomma nigricans.—In the earliest nurse cell examined the mitochondria are found in the perinuclear zone. They are granular and fine. In older stages of nurse cells (Fig. 22) the perinuclear zone spreads and has a tendency to disperse in the cytoplasm. In much older nurse cells only few granular and fine mitochondria are found scattered in the cytoplasm.

Nucleus and Nucleolus.

Cedria paradoxa.—It will be observed from Fig. 3 that the nucleolus of nurse cells show very great activity. The nucleolus takes up a deep stain in the acid fuchsin stain and iron-alum haematoxylin, in the former deep red and deep bluish black in the latter. In some of the cells the nucleolus has given rise to several nucleoli which lie inside the nurse cell nucleus. The activity is observed from a very early stage. A great number of the nucleoli are seen lying on the nuclear wall, some inside and some outside the nuclear membrane in the cytoplasm; many of the nucleoli have come far away from the mother nucleus and are lying in the nurse cell cytoplasm. These take up similar stain as those inside the nucleus but have not been observed to come out of the nurse cell into the general cytoplasm and flow down to the oocyte chamber along with the cytoplasmic current. It appears from the great activity of the nucleolus that these nucleoli have some definite function which could not be linked up in the present species. At least they have not been seen to be forming any reserve material in the nurse cell cytoplasm.

Rhogas plecopterae.—The nucleolus exhibits great

activity in giving rise to several nucleoli which lie inside the nurse cell nucleus. These have not been observed to come out. This activity is seen with bigger nurse cell nucleolus.

Enicospilus sp.—The nucleolus of nurse cells show great activity as a result of which several nucleoli are found inside the nucleus, staining bright red with acid fuchsin. Many of them come out of the nuclear membrane into the cytoplasm.

Dioctes gardneri.—The nucleolus of the nurse cells show rapid multiplication in giving rise to several nucleoli. Sometimes they get mixed up and form an uneven mass, staining deeply in acid fuchsin The nucleoli have not been observed to come out of the nucleus as seen in other species.

Trichomma nigricans.—The nucleolus stains bright red in acid fuchsin throughout the course of oogenesis. The nucleolus of follicle cells do not show any activity but in mature oocytes in some of the follicle cells two nucleoli are found inside the nucleus. The nucleoli of nurse cells perform great activity in giving rise to several nucleoli; they have been seen to lie on the nuclear membrane but they have not been observed to come out of the nuclear membrane.

Follicular Epithelium.

Cedria paradoxa.—The Golgi elements of the follicular cells resemble those of nurse cells and oocytes. They are vesicular in shape distributed about the nucleus. They have not been observed to filter into the ooplasm

The mitochondria of the follicular cells are fine and granular, very much similar to those of the nurse cells, and occuring all over the cytoplasm during the early stages. With a powerful oil immersion lens they are found to infilter into the ooplasm in an advanced egg in the late stages of oogenesis.

The nucleolus of the follicle cells in the late stage shows rapid multiplication (Fig. 3) and the nucleoli come out and lie in the cytoplasm. An interesting feature of the follicular cells is the appearance of a number of vesicular bodies at the base, close to the vitelline membrane and which resemble to early secondary nuclei of the egg proper. It will be seen from this figure that these secondary nuclei appear to possess a limiting membrane. The nucleolar particles are found to extrude into the cytoplasm of the follicular cells. These bodies recognised as secondary nuclei possess a deeply stained granule.

Rhogas plecopteræ.—The Golgi elements of the follicular cells resemble those of the nurse cells and oocytes. They are very few in number, distributed around the nucleus and come out into the ooplasm (Fig. 5).

The mitochondria of the follicular cells are granular and similar to those of nurse cells occurring all over the cytoplasm. It has been observed with the help of a powerful oil immersion lens that they infilter into the ooplasm. The mitochondria are uniformly distributed throughout.

The nucleolus of the follicular cells show great activity to a certain extent but they have not been seen to come out.

Enicospilus sp.—The Golgi elements of the follicular cells are like those of nurse cells and oocytes. For the most part they appear close to the nucleus and then scatter in the cytoplasm.

The mitochondria are granular throughout and are thinly scattered round about nucleus. In the late stage of oogenesis some of the follicle cells grow darker than others. The inclusions of the follicle cells infilter into the ooplasm in later stages (Fig. 19).

Dioctes gardneri.—The Golgi bodies of the follicle cells are vesicular with a distinct chromophilic rim. They

are few in earlier stages but later they scatter around the nucleus (Fig. 10, 11).

The mitochondria of the follicular cells are granular throughout the course of oogenesis. It may be observed here that the mitochondrial granules are comparatively bigger in size than the other species of parasites described above; they are not so fine and dust like as in others. The inclusions of the follicle cells infilter into the ooplasm in the older eggs (Fig. 14).

Oocyte.

Golgi bodies.

Cedria paradoxa.—The youngest oocyte contains vesicular Golgi bodies with distinct chromophilic rim as seen in the Ayoma preparation. The Golgi vesicles are found towards the periphery of the oocyte. In bigger eggs only small number of vesicles are found, mostly in the periphery amongst the yolk laden bodies (Fig. 2). In fairly well advanced oocyte (Fig. 3) the Golgi crescents are found in patches. The Golgi bodies are present in various forms—chains, beads, figures of eight, dumbbell shaped. The actual process of fission of Golgi bodies are also seen in the same figure which throws light on their method of propagation in the oocyte (Fig. 2).

Rhogas plecopteræ.—The Golgi bodies of the youngest oocyte (Fig. 7) contains a few bodies in series forming an arc on the nucleus; they finally spread uniformly in the ooplasm (Fig. 4). In the Mann-Kopsch preparation the Golgi bodies are seen to be vesicular in shape under the oil impression lens. With the growth of the oocyte the Golgi bodies are seen to increase in size slightly.

Enicospilus sp.—In the earliest oocyte a small number of Golgi grains are found lying near the nucleus (Fig. 12); then they increase in number occupying the juxtanuclear position. The Golgi bodies are vesicular in

shape which show distinctly chromophilic rim. With the growth of the oocyte the Golgi bodies also increase in number and size. In the bigger oocytes (Fig. 16) the Golgi vesicles and crescents are found and at places accumulation of vesicles forming apparently a net work is also seen. Ordinary fat is dissolved out in xylol and turpentine which leaves behind a vacuole. The amount of Golgi bodies of the oocyte is very limited in the earlier stages and in the advanced egg the vesicles and crescents fill up the egg leaving the centre free from inclusions.

Dioctes gardneri.—The Golgi bodies of the early oocyte are vesicular in shape with a distinct chromophilic rim; they are found around the nuclear membrane; then they spread to the periphery from the juxtanuclear position (Figs. 10, 11), leaving only a small number of Golgi bodies in the middle area of the oocyte The vesicles of this species are comparatively of bigger in size than the other species examined. In the mature egg the Golgi bodies are found to fill up the oocyte by increasing in number.

Trichomma nigricans.—In the earliest oocyte the Golgi bodies are found in an advanced juxtanuclear stage; they are both granular and vesicular Later from the perinuclear stage the Golgi bodies are seen to increase in number and shift to the periphery of the oocyte. A bipolar stage has been shown in Fig. 19. In a mature oocyte (Fig. 20) the Golgi bodies are found round the periphery of the egg; here crescents, vesicles and chains are seen. From some of the bigger Golgi rings the fat element has dissolved out with the treatment of turpentine and the ordinary fat bodies are represented by vacuoles.

Mitochondria.

Cedria paradoxa.—In the oocytes examined the mitochondria are found to be granular throughout. They are

fine and granular in the older eggs; their detection is rendered difficult on account of the considerable amount of the deutoplasmic bodies in such eggs.

Rhogas plecopteræ.—In the youngest oocyte (Fig. 6) the mitochondria occurs in the juxtanuclear position; then follows the perinuclear stage. The circumnuclear mass of mitochondria at first widens and becomes dense and finally disappears. The major portion of the mitochondrial contents spread along the two sides of the oocyte near the periphery. The rest of the cytoplasm in the middle is filled with the scattered mitochondria.

The mitochondria are granular and very fine in the early oogenesis; but later the filamentous type of mitochondria is observed in the older eggs intermixed with the yolk bodies and the granular mitochondria (Fig. 7). It appears that the filamentous mitochondria divide into granular forms because the filaments exhibit a beaded appearance.

Enicospilus sp.—The mitochondria of the early cocyte have been found in the perinuclear stage. They are granular in form throughout. From this zone the mitochondria spread. During later stages of growth the mitochondrial granules move towards the periphery and they get concentrated in this region. As the cytoplasmic current from the nutritive chamber flows through a direct passage into the oocyte chamber, it throws its inclusions to the periphery leaving the centre of the oocyte free from any inclusions (Fig. 17). In the mature egg mitochondria fills the entire oocyte.

Dioctes gardneri.—The mitochondria of the early oocyte (Fig. 13) is found to be in the perinuclear stage; then it spreads in the cytoplasm and a peripheral arrangement occurs. After this stage the mitochondria begins to increase in number towards the inner side of the developing oocyte. The inflowing cytoplasmic current from the nurse chamber to the oocyte throws the inclusions to the

periphery, keeping the middle portion free from any inclusion (Fig. 14). The mitochondria is found to be granular throughout.

Trichomma nigricans.—In the early oocyte the mitochondria is found as a cap on the nucleus in the juxtanuclear stage. The mitochondria is dust like and granular. From here it spreads and forms a perinuclear ring. In the older oocytes the mitochondria shift to the periphery of the oocyte where they begin to swell and transform into yolk bodies. The mature eggs contain very little of granular mitochondria as much of it is used up in building up reserve food material; only a small portion in the middle of the oocyte contains the granular mitochondria.

Nucleolar extrusions and Secondary nuclei.

Cedria paradoxa.—Like the nurse cell nucleolus the oocyte nucleolus exhibits from the very early stage great activity in giving rise to several nucleoli. The nucleoli come to lie on the nuclear membrane and then bodily pass out of the membrane, probably by rupturing the nuclear wall, and come to lie in the oocyte cytoplasm near the nuclear membrane. Here some of these extruded nuclei form the secondary nuclei surrounding the mother nucleus.

The very interesting feature of the cocyte is the origination of the so called secondary nuclei from the early stage. The extruded nucleoli are not very small in size and are clearly seen under the oil immersion lens. As these come out of the nuclear membrane they get surrounded by a clear space and the whole structure presents the appearance of a small vesicle with a single deeply staining granule in the centre. They gradually increase in size and fresh ones continue coming up. The secondary nuclei slowly spread out in the peripheral region of the oocyte. The single nucleolus of the secondary nuclei form many particles by growth and fragmentation; they

extrude in the cytoplasm like those of the head nucleus and give rise to a second generation of secondary nuclei. The appearance of these nuclei is associated with the yolk formation. A direct part taken by them has not been found out but an indirect part in the yolk formation is suggested for they have not been found disappearing from view even upto the late stage in oogenesis.

Rhogas plecopteræ.—The nucleolus of the early oocyte shows great activity in giving rise to several nucleoli but they do not come out of the nuclear membrane rapidly; only a small number is seen to have come out. Their exact role could not be determined in oogenesis because they do not appear to take any active part, but certainly, as the nucleolus shows activity, the nucleoli may indirectly aid in the elaboration of yolk bodies. Only in the late oogenesis in mature oocytes a small number of secondary nuclei are seen around the periphery, but they seem to be inert.

Enicospilus sp.—The nucleolus of the oocyte shows great activity from the very early stage as several nucleoli are found inside the nucleus. Several of the nucleoli are found near the nuclear membrane and some have extruded in the ooplasm in quite an early stage. These extruded nucleoli move towards the periphery and only in the advanced eggs they appear to take part in the formation of secondary nuclei (Fig. 17).

Dioctes gardneri.—It is observed that the nucleolus of the oocyte exhibits some activity though much less vigorously as reported above in other species. Even in the early oocyte the nucleoli are extruded from the nuclear membrane and lie in the ooplasm. In a still earlier oocyte (Fig. 13) the nucleus contains only one single deeply staining nucleolus. The extruded nucleoli form secondary nuclei. They shift to the periphery of the oocyte. The secondary nuclei persist in older oocytes as well and appear to take an indirect part in yolk formation.

Trichomma nigricans.—The oocyte nucleolus performs great activity, quite early in oogenesis. Several of the nucleoli are contained inside the nucleus from which they have not been seen to come out of the nuclear membrane but it is quite probable that they indirectly aid in the elaboration of food material (Fig. 22.)

VITELLOGENESIS

Pedria paradoxa.

Fatty yolk.—The fatty yolk formation starts in older stages of oocyte; younger stages show no fat bodies except scattered few vesicular Golgi elements with thick argentophil black rim. From the earliest stage the oocyte is seen to contain many greyish yolk bodies; in the advanced eggs these bodies are found intermixed with the fatty yolk bodies; the quantity of the former is lesser than the latter; probably these are cytoplasmic yolk bodies.

Albnminous yolk.—The albuminous yolk bodies are formed as a result of direct swelling of granular mitochondria which take a deep pink stain in the acid fuchsin in the chrome-osmium preparation. But the majority of the granular mitochondria remain granular throughout and the exact stage of the swelling of mitochondria is not seen though swelling mitochondria are visible from quite an early stage of oogenesis.

Further, from the very earliest stage of growth of oocyte and onwards the ooplasm carries numerous extruded nucleolar particles thrown off by the head nucleus. It appears that all the extruded nucleoli are not used up in the formation of secondary nuclei as the greater number remain freely scattered in the ooplasm; these at the time of yolk formation grow up into proteid bodies inside a clear space in the cytoplasm, but in the absence of convincing results of Bouin preparations it cannot be confirmed because of the difficulty of distinguishing between

the origination of the two kinds of yolk bodies, mitochondrial and nucleolar.

Rhogas plecopteræ.

Fatty yolk.—The amount of fatty yolk is less in this species; only a small number of swollen Golgi bodies have been seen (Fig. 5); with the treatment of turpentine the fat dissolves away and leaves behind a chromophilic rim, confirming the origin of the fatty yolk. It also suggests that the chromophilic rim of the vesicular Golgi bodies elaborates fat inside the chromophobic area with the materials derived from the ground cytoplasm; thus it will be observed that the fatty yolk is formed probably not as a direct metamorphosis of the Golgi elements but in the manner suggested above. The ordinary fat bodies formed in the ground cytoplasm without the intervention of any inclusion is quickly dissolved out and is only represented by several vacuoles.

Albuminous yolk.—The albuminous yolk bodies are formed as a result of direct swelling of mitochondria (Figs. 7, 8). It is quite probable that as the growing oocyte is assisted by the inclusions of the nurse cells through a direct passage as occurs in the late oogenesis, or by way of infiltration the mitochondria of the nurse cells may also swell to form yolk bodies in the oocyte although they have not been seen to be so transformed while in the nurse chamber. The yolk formation begins at a late stage in oogenesis.

Enicospillus sp.

Fatty yolk.—The Golgi bodies are seen to take a direct part in the formation of the fatty yolk; but their amount in the oocyte is not much. The Golgi rings from which fat has dissolved out after the treatment of turpentine may also be seen. The fatty yolk formation starts in slightly advanced oocyte. The ordinary fat bodies are represented by vacuoles.

Albuminous yolk.—The albuminous yolk bodies are formed as a result of direct swelling of mitochondria; but majority of the mitochondria remain granular throughout the course of oogenesis (Fig. 18). In this species the extruded nucleoli can not be said to take any active part in the yolk formation as they are few in number. The granular mitochondria in the advanced eggs form the yolk bodies with the aid of the ground cytoplasm; much of the contents of this egg is filled with the ordinary fat vacuoles and some granular mitochondria.

Dioctes gardneri.

Fatty yolk.—The Golgi bodies take a direct part in the formation of fatty yolk bodies which process starts quite early in the oogenesis (Fig. 10). As the oocyte is treated with turpentine the chromophilic rim of the vesicle is left behind from the interior of which the fat has been dissolved out. In an advanced egg plenty of fatty yolk bodies are seen. The ordinary fat bodies are represented by vacuoles.

Albuminous yolk.—The albuminous yolk bodies are formed as a result of direct swelling of mitochondria in the later stages of oognenesis. They are mostly to be found in the periphery of the oocyte. For the most part the mitochondria remain granular. In this species also the amount of the extruded nucleoli is less and as such the part played by them in the formation of yolk bodies may be considered ineffectual.

Trichomma nigricans.

Fatty yolk.—The amount of fatty yolk is less in this species. The mature egg (Fig. 20) shows some of the vesicular Golgi elements and bigger ringed vesicles from which fat has dissolved out with the treatment of turpentine. They occur mostly in the periphery of the oocyte.

Albuminous yolk.—The albuminous yolk bodies are formed as a result of direct metamorphosis of mitochon-

dria. The yolk formation begins quite early in oogenesis; in Fig. 21 swelling of some of the granular mitochondria are seen; it appears that the yolk formation starts from the periphery towards the interior (Fig. 22). In mature egg all the stages of the yolk bodies are very well seen; these have taken bluish stain in the iron-alum haematoxylin. In the ripe egg the granular mitochondria form a very thin layer in the periphery and the entire egg is filled with the yellow yolk bodies. These yolk bodies which have not responded to the fuchsin colour are probably cytoplasmic yolk bodies. It will be observed that this kind of yolk body has been referred above in three species of parasites, namely *Cedria paradoxa*, *Enicospilus* sp. and *Trichomma nigricans*, but it requires further confirmation till more females are available for examination.

Centrifuge Experiment.

Cedria paradoxa, *Rhogas plecopterœ and Trichomma nigricans* (Fig. 24).—A complete separation of the cytoplasmic inclusions into various layers did not take place in respect of their specific gravity. The centrifugal pole in all the species shows that the mitochondria is the heaviest of the egg inclusions; here the swollen stages of mitochondria are also seen; further, the yolk bodies show also a direct relationship with the mitochondria for they also occupy the centrifugal pole. Although the results of Bouin preparations are missing the examination of slides of the centrifuged eggs in the chrome-osmium preparation leaves no doubt as regards the origin of yolk bodies in view of the observations that all stages are seen from granular to swollen mitochondria and finally into yolk body. The centripetal pole is completely occupied by the ordinary fat vacuoles. *Trichomma nigricans* shows the yellow yolk bodies (viz., the cytoplasmic yolk bodies which have not responded to the fuchsin colour)

occupying the centrifugal pole. As a complete separation has not taken place into various layers no middle layer is seen which is thinly filled with the granular mitochondria and few yolk bodies, evidently in the act of settling towards the centrifugal pole of the egg. Similar observations are found with the follicle and nurse cells whose nucleolus and nucleoli aggregate at the centrifugal pole, facing which the granular mitochondria also collect in the cytoplasm.

Germcell Determinant.

Cedria paradoxa, *Enicospilus* sp. and *Dioctes gardneri* show at the posterior end of mature eggs a deeply staining body. In *Cedria paradoxa* this body has appeared in the chrome-osmium preparations stained with both acid fuchsin and iron-alum haematoxylin, red in the former and bluish black in the latter; it has also appeared in the Bouin preparations stained with iron-alum haematoxylin. In *Enicospilus* sp. it is more distinct and elongated in the champy preparation stained with acid fuchsin. In *Dioctes gardneri* the body has been seen only in the Mann-Kopsch preparation treated with turpentine; it is seen greyish in colour. Under the examination of high power oil immersion lens it has shown no inclusions of any kind but shows clearly a differentiated cytoplasm which has taken a deeper colour.

Chatterjee (1938) in *Apanteles machaeralis* Wlkn., found a round body at the posterior pole of the mature egg stained deep bluish black in the Bouin preparation and a similar body in *Apanteles malevolus* Wlkn., in the champy preparation stained with acid fuchsin. These also do not show any of the inclusions but appear to be only a specialised ooplasm. The body referred resembles the familiar germ cell determinant of the various authors (Gatenby, Sylvestri and Hegner in Coleoptera and Hymenoptera).

DISCUSSION.

Nurse cells.

The nurse cells are recognised as nutrimentary in function and aid in the growth of the egg by the contribution of substances elaborated by them (Paulke 1900, Snodgrass 1925). Peacock and Gresson (1928) reported the infiltration of the nurse cell cytoplasm into the ooplasm in Tenthridinidae and also showed the passage of nurse cell nuclei into the egg; finally, the entire cytoplasmic mass and the nuclei of the nutritive chamber pass into the oocyte. Gross (1903) holds the view that the nurse cell nuclei are absorbed only partially or not at all. Chatterjee (1938) has reported the gradual disappearance of nurse cells whereby the nurse chamber degenerates and the entire content is finally used up in nourishing the developing oocyte. In the present investigation on the parasitic species signs of absorption of nurse cells by the developing oocyte is very well seen in some of the species where a direct communication between the two chambers takes place. But the majority of workers have not mentioned this phenomenon in their material, *i.e.*, Gatenby (1920), Nath (1924, 1929), Payne (1932), Nicholson (1921), Hosselet (1931), Nusbaum-Hilarowicz (1917), Loyez (1913), Gunthert (1910), Govaerts (19 3), Dederer (1915), Hegner (1915), Hogben (1920) and Srivastava (unpublished)

Several workers have reported the passage of nurse cell cytoplasm into the egg—Weiman (1910), Nusbaum-Hilarowicz (1917), Goaverts (1913), Bhandari and Nath (1930), Nath (1924), Dederer (1915) and Hegner (1915). The exact nature of the granules flowing from nurse chamber to the oocyte has been correctly recognised only by a few, *i.e.*, Nusbaum-Hilarowicz (1919), Bhandari and Nath (1930), and Goaverts (1913). Srivastava (1934),

Shyam Mohan and Bhattacharya (1935) and Chatterjee (1938) have shown that along with the cytoplasmic stream of the nurse chamber a good amount of nurse cell Golgi bodies and mitochondria pass into the ooplasm and they infilter as well while the partition membrane is intact. Srivastava (unpublished) has mentioned some variation in this connection; for instance in *Cybister confusus* the inclusions flow down from the nurse cell to the oocyte through the interconnecting bridge and with the disappearance of the bridge no transference takes place; but in the lepidoptera *Danais chrysippus* the transference does not stop even after the disappearance of bridge and is continuous through a secondarily established connection; in the hymenopterous forms he finds that both the inclusions, Golgi bodies and mitochondria, are carried along with the nurse cell cytoplasm into the oocyte. But Gresson (1929) working on the group Hymenoptera (Tenthredinidæ) also reported the absence of the inclusions in the cytoplasmic stream. My present work on the parasitic species of forest insects have shown that the inclusions from the nurse chamber infilter into the oocyte chamber.

Follicle cells.

The function of follicle cells are protective and nutrimentary; it is a recognised fact It is only in the recent days that the processes analogous to the passage of the cytoplasmic inclusions of the nurse cells have been discovered with regard to them and it is held that they too like the nurse cells send their inclusions to the developing oocyte. In the year 1925, two cytologists, Bhattacharya and Brambell discovered that the Golgi bodies of the follicular cells, in the tortoise and fowl respectively, infilter into the ooplasm. The Japanese cytologist Ikeda (1928) observed the infiltration of follicular Golgi bodies and mitochondria into the oocyte in some

birds. Now this phenemenon is gaining support from the majority of the modern workers, both in the vertebrates and invertebrates (Bhattacharya and others). Recently Gosta Jagersten (1935) has questioned the reality of so wide phenomenon of infiltration and the present writer thinks that it will be useful if Jagersten makes a careful re-examination of his slides. The methods of infiltration vary in different animals and can be studied only by the specific methods. In the present investigation further support on the phenomenon of infiltration is obtained with the osmic techniques. Singh (1938) doubts that the silver techniques are not specific as a result of his study in the stickleback.

In one species *Enicospilus* sp. under examination (Fig. 17) some of the cells of the follicular epithelium of an advanced oocyte show darkening; it was not seen in any other species. This kind of follicular darkening has also been observed by Gresson (1928), Srivastava (1934, and thesis 1937 unpublished). This phenomenon is of wide occurrence in the vertebrate eggs. Loyez (1905) thinks that they are fixation artefacts. The present writer is of opinion that the cells represent a degenerating stage.

In some of the parasitic species *Trichomma nigricans* and *Rhogas plecopteræ* (Figs. 9, 21, 22) the follicular cells elongate and protrude into the developing oocyte or they quickly multiply and become two layered and appear to crush the growing oocyte. A similar crushing of the growing oocyte by the follicle cells has been previously recorded in *Apantelels machaeralis* (loc. cit.). Srivastava has also observed this in some insects.

Golgi Bodies.

The structure, behaviour and function of Golgi apparatus in a developing egg is still open to serious criticisms and unanimous opinion has not been arrived at by

the various workers in the field of cytology. Gatenby's (1938) conception of the structure of the Golgi apparatus is that of a chromophilic material (argentophil or osmiophil) which very often encloses a non-staining or chromophobe material. He opines that the chromophil substance may exist in the form of vesicles, batonettes or scales and can be seen *intravitam*. According to Payne the methods which demonstrate Golgi apparatus are not at all specific and Jägersten (1935) shares his opinion with that of Payne.

Nath (and collaborators 1924—31) believes that the Golgi bodies have got the shape of a vesicular type which consists of a chromophilic outer rim and chromophobic interior. Nath's conception about the Golgi apparatus is that in the living condition it appears as vesicles their homogenous form being due to over-impregnation. He finds in this kind of interpretation and form of Golgi elements a very satisfactory explanation of fatty yolk, to be discussed later. Nath's school is of opinion that all other forms of Golgi bodies in the eggs are artefacts. In support of this they mention that a crescent may be produced by the partial blackening of chromophilic rim or may be that the appearance is merely an optical illusion. Gatenby and Nath hold practically the same view regarding the structure of the Golgi apparatus. The present writer working with insect parasites also finds the Golgi bodies as vesicular with the characteristic duplex nature.

Gatenby (1938) says that the chromophil material of Golgi apparatus is of liquid nature and the chromophobe material is non-fatty and probably of the nature of protein; and the chromophobe material can not always be made out especially in the egg cell. Singh and Boyle (1938) have found by ultracentrifuging the oocytes of the stickleback that it is only osmiophil or argentophil substance of the Golgi apparatus that moves and collects at

the centripetal and below the fatty layer. Similar results have been obtained by Normington (1937), Daniels (1938) and Singh (1938) by using the ultracentrifuge. Recently Boyle (1938) in the neurones of *Helix aspersa* has shown that the chromophobe part of the Golgi apparatus is fatty in nature as it is stained with Sudan IV. Subramaniam (1937) and others have shown that the neutral red stains the chromophobe portion of the Golgi vesicle. Thus it may be quite possible that the chromophobe part is not the true apparatus as described by Golgi whereas argentophil or osmiophil part is the real apparatus. The present writer has found that as the ovarioles are treated with neutral red for the demonstration of 'vacuome', in the same slide on the introduction of 2% osmic acid, the Golgi bodies showing the duplex nature appeared and in some the interior of these vesicles, the chromophobe part, absorbed the neutral red colour. Similar observation was also made with *Apanteles machaeralis* (1938). The present writer thinks that the conception of Gatenby regarding the shape of the Golgi apparatus is quite reasonable, for he says that the chromophil substance may exist in the form of batonettes, vesicles and scales and it affords an explanation of the net-work like structures found in the vertebrates. The second part of the conception of Gatenby that the chromophil substance may exist in various forms supports Nath's conclusions that it may have to do with the function it executes, i.e., the deposition of the free fat inside the Golgi vesicle.

The objections raised by Harvey, Schlottke and Jagersten against Nath and Greeson that they have described the developing fat bodies as Golgi elements has been discussed recently by Nath (1934). He says that according to the biochemists lipin forms fat in the cell and the lipoidal Golgi elements of eggs may aptly be described as "developing fatty droplets." Among the recent supporters of the view that the lipoidal Golgi apparatus

form fat in the cell are Harvey, Greeson and Bell. Jagersten's (1935) dogmatic assertion that in all the cases the Golgi elements described by Nath are not Golgi elements at all but fat bodies is unsound for he must know that fat bodies are not preserved in the silver preparations on which most of Nath's descriptions are based.

In oogenesis striking example of the growing of Golgi bodies is found in the eggs of *Testudo graeca* described by Bhattacharya (1925). Here the Golgi bodies are seen dividing and producing chains. This affords strong evidence that the Golgi bodies in oogenesis increase by fission. Various workers have found this to be so. The present writer has observed the Golgi bodies actually in the process of fission in *Cedria paradoxa* in the Ayoma preparations (Fig. 2).

The fact that the Golgi elements are not passive is borne out by the works of Gatenby (1917 and onwards) and Bowen. Since then reports have accumulated that during the mitotic processes they are sorted out and distributed evenly to the daughter cells—Gatenby and Ludford (1921), Bhattacharya (1925)), Payne (1932) and others. Gatenby (1929) has shown their distribution to the ectoderm, endoderm and mesoderm cells of the gastrula of *Limnaea*. Further, the Golgi elements have been seen in the living animal cells without the aid of any reagent—Bhattacharya and Das (1929), Rau and Brambell (1925), and Obrien and Gatenby (1930). They follow orderly behaviour in dictyokinesis. This is in itself a sufficient proof of the view that the Golgi apparatus does exist in animal cells. Thus the views of Allen and Walker (1929), Schlottke (1931), Tenant and Gardiner and Smith (1931) fall to the ground. The present writer has observed the Golgi elements in the living ovariole of parasitic species without any reagent; they were observed as vesicular with faint boundary line and an opaque interior, dancing dur-

ing examination; further these very bodies with the application of osmic acid became fixed and showed their duplex nature and crescent shape.

The views of workers in oogenesis about the function of Golgi bodies is still far from reaching unanimity. Prominent investigators on this branch of cytology are Gatenby, Ludford, Brambell, Nath and Bhattacharya. They are all in favour of the view that these cell constituents directly or indirectly give rise to fatty yolk.

Harvey (1925, 27, 29, 31), Hibbard and Parat (1927, 28), Hibbard (1928) and Steopoe (1927) advocate a different function to the Golgi apparatus, *i.e* , that of aiding in the formation of the albuminous yolk. It appears that all these workers have been led away by Hogben's theory of yolk formation. He observed yolk being deposited in the chromophobic part of the Golgi apparatus and considered the proteid yolk formation as a condensation into droplet from the Golgi material synthesised by mitochondria, the plasmosome and ground cytoplasm. Thus, according to Harvey the Golgi bodies have only an indirect role to play n oogenesis in connection with the synthesis of yolk.

Subramaniam (1936) opines that the primary function of the Golgi apparatus during oogenesis is the production of intracellular enzymes and that yolk and fat are secondary products resulting from the action of these enzymes.

Gatenby (1938) says "If we adopted the view that one function, at least, of Golgi apparatus of metazoan cells was to concentrate by partial dehydration, the secretory material originating either from the ground cytoplasm or from mitochondria, this would bring into line the duplex contractile vacuole and metazoan Golgi apparatus . . . . . ; while there are certain difficulties, this hypothesis would fit in well with what is known of the behaviour of the Golgi apparatus in metazoan gland cells and eggs at least".

Gatenby's earlier conception about the function of the Golgi bodies in oogenesis was the origin of fat but this was

in the days when neither ultra-centrifuge nor the combined method of Ayoma and Sudan IV was available. But now with more efficient methods of staining and use of ultracentrifuge, all the previous works of Gatenby, Ludford, Nath and collaborators, Brambell, Bhattacharya and his pupils, Gresson, Subramaniam, Hirschler, King, Woodger and others, will be subject to reinvestigation in order to arrive at a correct view as regards the function of Golgi apparatus. Gatenby (1937) says "For oogenesis studies and the Protozoology . . the ultracentrifuge is indispensible . . . . . ", Singh during recent years has spread his work on Protozoa, Birds and Fishes and believes that the "problem of neutral red cytology and vitellogenesis and the neutral red cytology in Protozoa can not be solved unless one uses the ultra-centrifuge."

The present studies on the insect parasites show that the function of Golgi bodies is related to fatty yolk formation, relying upon the observations made by silver and osmic preparations. As yet our laboratory has not been properly fitted up with the ultracentrifuge apparatus. It is hoped, however, that in the near future results on the new line of investigation will be forthcoming when the current view of Professor Gatenby may be examined more thoroughly.

Mitochondria.

Like the Golgi bodies, mitochondria are as well polymorphic in nature—spherical, granular, rod-shaped, crooked, filamentous and in the form of a close net work (Gatenby, Nath, Bhattacharya, Greeson, Aykroyd and others). The present writer has found in the forest parasites that the mitochondria are granular throughout except in *Rhogas plecopteræ;* in the latter species filamentous type fills the mature eggs and shows beaded strings evidently forming granular forms. In the vital experiments the filamentous type has been observed in *Cedria paradoxa,*

Dioctes gardneri and *Trichomma nigricans* to stain with the dye Janus green B.

The existence of mitochondria in cells is almost universal except that according to Cowdry (1921) their existence is doubtful in Bacteria and non-nucleated red blood corpuscles.

The origin of mitochondria has been full of controversies. The chondriome theory finds no strong supporters (Gatenby 1920, Gatenby and Woodger 1920 and Nusbaum-Hilarowicz 1917). In some cases the mitochondria have not been detected in the early oogonia and earliest oocyte (Gatenby in *Saccocirrus* 1922, Gardiner in *Limulus* 1927, Nath and co-workers in *Lithobius* 1924 and *Ophiocephalus* 1931). This would lead to their *de novo* origin in the cytoplasm, or it may as well be thought that the workers failed to detect mitochondria for some practical defect in technique. But Gatenby has shown their distritution to the cells of germ layers of *Limnae*, thereby proving their continuity from generation to generation.

In the species of insects that have been studied here the mitochondria have been found in the earliest stages of egg though on account of their very small size their examination is rendered difficult. The fact that the mitochondria have been observed to increase in quantity from the earliest to later stages suggests that they grow and multiply and fill up the egg. Their detection becomes difficult in the yolk laden mature eggs.

About the function of mitochondria in oogenesis there is a good deal of variation in different animals. The mitochondria in insects with few exceptions have been shown to remain granular throughout the course of oogenesis and are reported to take no part in vitellogenesis The present work leaves no doubt as to their importance in yolk formation. They are as important as the Golgi bodies in the elaboration of reserve food material.

Nucleolar extrusions.

The phenomenon of nucleolar extrusions is of wide occurrence both in the vertebrates and invertebrates (Gatenby, Nath, Bhattacharya and others). Payne (1932) has failed to obtain any evidence of nucleolar extrusions in his studies on insect ova though he has used the chrome-osmium mehods which very well show this phenomenon. Jägersten (1935) interprets the extruded bodies not as nucleolar extrusions but as having been elaborated independently in the ground cytoplasm. It is very difficult to accept his views in the face of clear cut processes of extrusions available from the works of various authors and the present study on forest parasites.

Secondary Nuclei.

Thus far, the accessory or secondary nuclei have been recorded in the oocytes of Coleoptera, Hemiptera, Lepidoptera, Diptera, and Hymenoptera. The following broad facts have been compiled by Mukerjee (1930) from the results of several investigators on accessory nuclei :

(1) They have been found in the cortical ooplasm of the oocyte or concentrated around in germinal vesicle.

(2) Their origin is uncertain.

(3) They have the power of growth, *i.e.*, of increasing in size.

(4) They stain with basic dyes, with the exception of Feulgen's to which they react negatively.

(5) They have the power of reproduction which is accomplished only by amiototic budding.

(5) They always disappear before maturation period.

Srivastava (unpublished) has worked on the cytoplasm of several orders of insects and has given an exhaustive historical account of secondary nuclei, and also his own

observations. He finds that they are confined mosly to the order Hymenoptera though they have been reported from other orders of insects as well. His conclusions are that they contain no chromatin as determined by the Feulgen's technique; they arise from the nucleolar extrusions in oocyte and that they take part in vitellogenesis.

The present writer's observations on the secondary nuclei of the Braconidae and Ichneumonidae are entirely based on the chrome osmium preparations stained with acid fuchsin. As already stated, the secondary nuclei arise from the extrusions of the oocyte nucleolus; evidence of the secondary nuclei formed from extrusions of follicle cells being available only from *Cedria paradoxa*. These appear quite early in oogenesis and are again found in the advanced oocytes, specially on the periphery They have been seen even in the advanced eggs containing yolk bodies. The nucleolus of some of the secondary nuclei has been shown to consist of a number of nucleoli which remain within the primary secondary nuclei; this is observed in late oogenesis. Apparently, evidences show that these come out and take part in the formation of the second generation of secondary nuclei; this is also seen in the secondary nuclei of follicle cell origin. All of the oocyte nucleolar extrusions do not take part in the formation of the secondary nuclei. Some of them disappear later in oogenesis in the ooplasm and some take part in the formation of yolk bodies as seen in *Cedria paradoxa* and *Enicospilus* sp.

The observations made above show that secondary nuclei definitely grow in volume during oogenesis and that they indiretly aid in the formation of yolk bodies, because they have been seen to occur with the yolk bodies and do not appear to disappear from view upto a late stage in oogensis. Similar suggestions are put forward by Gatenby (1920) in *Apanteles glomeratus*, Loyez (1908), Chatterjee (1938) in *Apanteles machaeralis* and Srivastava (unpublished) in *Scolia quadripustulatus*.

Vitellogenesis.

In the present investigation the author has found three kinds of yolk bodies in the egg. One is the fatty yolk formed under the influence of Golgi bodies, the other the mitochondrial yolk bodies (albuminous yolk) formed by the direct transformation of mitochondria and a third formed independently in cytoplasm known as cytoplasmic yolk. The Golgi origin of fatty yolk has been confirmed by the method of fat extraction with turpentine. With prolonged exposure to turpentine the ordinary fat is completely dissolved out leaving behind the chromophilic rim. In cases where the action of turpentine has been only for a short time the fat appears as black spherical body of various sizes. It may be said that the Golgi fatty yolk bodies are not abundant but they are present in sufficient amount. The fat bodies become red in Sudan III and Scharlack R and are found in abundance in mature eggs and less in quantity in the earlier stages.

In *Cedria paradoxa* the formation of fat under the influence of Golgi bodies by swelling is very well seen in the Ayoma preparation in later stages; the osmic acid failed to react in this which could not be ascertained definitely at present but by treating the living ovariole with 2% osmic acid the Golgi duplex and crescents appeared after a long time. In the rest of the parasitic species fatty yolk formation has been studied with the Mann-Kopsch technique and by subsequent treatment with turpentine. It has been observed that the fat bodies in *Enicospilus* sp. is more resistant to turpentine than those of *Dioctes gardneri* and *Trichomma nigricans* where quickly the fat decolorises.

The writer believes that the Golgi bodies here are taking a direct role in the formation of fatty yolk; but he interprets the formation in the light that it is the chromophilic rim which serves as a directive influence on the ground cytoplasm of the egg. It elaborates yolk inside the chromophobic area and thus the metamorphosis of the

Golgi bodies takes place in the formation of fatty yolk which is revealed by turpentine; it dissolves out the weak fat from the chromophobe substance.

The second type of yolk bodies present in the parasitic species is the albuminous yolk. They are directly transformed by the direct swelling of granular mitochondria into yolk bodies. They have been studied in the chrome-osmium preparations stained with acid fuchsin and iron-alum haematoxylin; in the former both the mitochondria and yolk bodies stain red and in the latter bluish black. The quantity of yolk bodies is abundant in the eggs, specially in the mature eggs where it completely fills the egg and the detection of the very fine mitochondria is very difficult The swelling of mitochondria starts quite early in oogenesis It appears that the albuminous yolk bodies first make their appearance from the periphery and gradually fill the egg inwards. In *Trichomma nigricans* it appears that among the albuminous yolk bodies in the older oocytes some remain golden yellowish in colour and do not respond to the stains but the peripheral yolk bodies take up the fuchsin colour.

The type of yolk body which is not coloured distinctly either by acid fuchsin or by iron-alum haematoxylin is the third type which the present author believes is formed from the cytoplasm directly or indirectly. In the presence of great nucleolar activity throughout oogenesis, part of the extruded nucleoli may help indirectly in the formation of yolk bodies. A part of them form the secondary nuclei while the remaining disappear in the ooplasm in the late stages; an indirect aid is thus given by the secondary nuclei in the formation of yolk bodies on account of their presence upto a late stage of oogenesis.

Gatenby (1920) in *Apanteles glomeratus* finds a complete absence of fat droplets and was unable to demonstrate the Golgi apparatus by osmic or silver methods although yolk appeared at the periphery among the mass of mitochon-

dria. He found absolutely no evidence regarding the metamorphosis of mitochondria into yolk bodies. Greeson (1930) has recently worked on the yolk formation of Tenthridinidae and finds that the fatty yolk is formed from the Golgi bodies and albuminous yolk from nucleolar extrusions. Payne (1932) working on the cytoplasm of insects concludes that the cytoplasmic inclusions do not have any part to play in the yolk formation and that they are formed from the ground cytoplasm. Srivastava (unpublished 1937) reports complete absence of fatty yolk in some of the non-parasitic species of insects, namely *Polistes*, *Vespa* and *Scolia*, and speaks of the formation of yolk bodies from the nucleolar extrusions.

But the present writer's findings in forest parasites show that the fatty yolk is present and is formed by the intervention of Golgi bodies and the albuminous yolk by swelling of mitochondria. It may be mentioned here that the earlier stages of forest parasites are carnivorous and the female adult parasites, as necessity arises, prefer to suck host larvae (Chatterjee 1939, 1943).

A direct transformation of the Golgi bodies into fatty yolk bodies is claimed by most of the workers (Gatenby, Nath, Bhattacharya, Dutta, Brambell and others). Voinov (1925) in *Gryllotalpa* showed Golgi corpuscles producing fatty yolk spheres. Hosselet (1930, 1931) concluded that the fatty yolk elements arise by direct transformation of the chondriosomes whose fuchsinophility decreases as the osmiophility increases. Greeson (1931) in *Periplanata* described the origin of fatty yolk spheres by the Golgi elements. Payne (1932), on the other hand, finds no evidence which could lead him to the conclusion that the Golgi bodies directly or indirectly are concerned in either yolk or fat formation. Singh and Boyle (1938) say "As far as we know, in oogenesis, fat is always secreted by the ground cytoplasm and never by the Golgi bodies as has been seen in our present investi-

gation (vitellogenesis of *Gasterosteus*). In *Amoeba proteus* . . . there is no Golgi apparatus present, but still there are large quantities of sudanophil fat. It is a very clear case of the origin of fat from the ground cytoplasm. Similar results have been obtained by all recent workers . . . It might also be suggested that the function of Golgi apparatus may be the nature of a catalytic agent in oogenesis. It probably helps in the formation of the material originating from the cytoplasm or mitochondria." It may be noted that these recent results have been arrived at with the aid of ultracentrifuge and combined method of Ayoma and Sudan IV.

There are many workers who completely deny the existence of any intimate connection between the Golgi bodies and the process of fatty yolk formation (Harvey, Weiner, Jagersten). But most of the cytologists opine that fatty yolk bodies are derived directly or indirectly from the Golgi bodies; some would derive it from the mitochondria, some from extruded nucleolar body and some from the ground cytoplasm without the intervention of any cytoplasmic elements.

From a review of the literature on the formation of proteid yolk bodies in the various groups of animals it is found to be formed in relationship with the (a) Golgi bodies, (b) mitochondria, (c) nucleolar extrusions, (d) 'vacuome', (e) secondary nuclei and (f) independently in the ground cytoplasm. The present writer has found in the forest parasites that the mitochondria take a direct part in the yolk formation and the latter is indirectly aided by nucleolar extrusions and secondary nuclei.

The 'Vacuome'.

The present author thinks that the recent findings on 'vacuome' have brought an end to Parat's 'vacuome' theory, which, since the inception of the theory, engaged the cytologists during all these years for a satisfactory

solution of the problem. All have found that the Golgi apparatus and 'vacuome' are not identical, for they exist side by side. Further, the Golgi elements can be seen in the living condition (Bhattacharya and Nath collaborators, Payne, Gatenby and others). A near answer to Bhattacharya and Srivastava (1935) " . . . Are the neutral red vacuoles the homolouges of the classical Golgi bodies as revealed in fixed tissues?" is to be found in the observations of the following two workers.

Gatenby (1937) writes "In animals many cells segregate neutral red both supra-vitally and intravitally. In some cases the neutral red dissolves or is segregated within pre-existing granules or vacuoles. . . . , in others, the globules and net are neo-formations. . . , in still others, both types appear in one cell and finally by some interaction between the dye stuff and the protoplasm insoluble bodies called 'Krinom' (Chlopin) become formed . . . . . Volutin granules, fuchsinophile granules, chromatic and metachromatic granules, prezymogen granules, single granules in insect and other spermatids are only a few of the assemblage of formed bodies which becomes red in this dye. Some of these bodies certainly go black in osmic and silver methods as well and it appears to us just as unjustfiable to call them Golgi apparatus as to classify all granules which stain vitally in neutral red as 'vacuome' ". Gatenby is not prepared to promote the 'vacuome' to the status of a definite and constant cell inclusion like the Golgi bodies and mitochondria and proposes to drop the word.

Singh and Boyle (1938) say "We think that the 'vacuome' in oogenesis, at all events, does not exist. . . . . it is sufficient to say. . . . as has been shown by using ultracentrifuge, no 'vacuome' exists in animal cells. The classical demonstration of Parat's 'vacuome' in the *Chironomus* salivary gland cells has been recently shown to be an artefact. . . typical crescentic Golgi

bodies exist in salivary gland cells of *Chironomus* larvae; these were overlooked by Parat."

But the observations of Subramaniam (1937) and Boyle (1937) throw light on the enzymic nature of Golgi bodies which the latter secrete inside the chromophobic part. That is why the chromophobic parts are not always visible in the ordinary living cells. The presence of these is revealed by the application of neutral red and the latter, according to the biochemical evidence forms compounds with enzymes These observations require to be confirmed.

SUMMARY

1. The cytoplasmic inclusions in the oogenesis of forest insect parasites have been worked for the first time. The following species form the subject matter of the present investigation.—

(a) *Cedria paradoxa* Wlkn. (b) *Rhogas plecopterae* Chatterjee. (c) *Dioctes gardneri* Cush (d) *Trichomma nigricans* Cam. (e) *Enicospilus* sp.

2. The modern technique as given in the latest edition of Vade Mecum (1937) has been followed.

3. The noteworthy results obtained are the presence of fatty yolk derived from the Golgi bodies, albuminous yolk produced by the direct swelling of mitochondria and cytoplasmic yolk probably formed indirectly from the nucleolar extrusions and secondary nuclei. The results are interesting in the fact that all the three methods of yolk formation mentioned have not been reported in Hymenoptera, within the knowledge of the present writer.

4. Nurse cells in the earlier stages send their inclusions by infiltration and in the later stages establish a direct passage with the oocyte chamber; through this path there is a flow of cytoplasm from the nurse chamber and along with the stream the inclusions also are carried to the oocyte. The follicle cells also send their inclusions most probably by infiltration which form a little denser layer in the periphery of the oocyte where infiltration occurs

5. Due to some physiological disorder in the eggs, sometimes the follicle cells destroy the developing oocyte either by growing longer and protruding into the oocyte or rapidly multiplying and forming layers of follicle cells. These are cases of atresia.

6. The oocyte nucleolus forms several nucleoli, some of which go to form secondary nuclei, some indirectly aid in the yolk formation and others disappear in the ooplasm.

The nurse cell nucleolus shows still greater activity as a result of which in the advanced stage the nucleus is packed with the nucleoli. Convincing observations are not forthcoming whether they perform any definite function though in some they are extruded into the cytoplasm. The follicle cell nucleolus shows the least activity.

7. The Golgi bodies are found to be vesicular and mitochondria as granular; the Golgi bodies are also present in the forms of crescents, the duplex nature, beads, chains and grow by fission as seen in *Cedria paradoxa*. In *Rhogas plecopteræ* filamentous mitochondria have been observed in the late stage of oogenesis. They appear to give rise to granular forms.

8. The separate identity of the Golgi bodies, mitochondria and yolk bodies have been established in the living ovariole without any stain.

9. The 'vacuome' and Golgi bodies have been seen to have no connection with each other as revealed by neutral red.

10. Janus green B stains mitochondria. Both granular and filamentous types have been observed.

11. The fat is stained in Sudan III, Scharlach R and Sudan IV.

12. In the electrically centrifuged eggs the mitochondria and albuminous yolk bodies go towards the centrifugal pole and the Golgi bodies and fatty yolk occupy the centripetal pole.

13. A graphic representation of yolk formation in the forest insect parasites is given below :—

Oogonium	*Full grown oocyte.*
a. Golgi apparatus	i. Definitive Golgi apparatus. ii. Golgi fatty yolk.
b. Mitochondria	i. Definitive mitochondria. ii. Swollen mitochondria. iii. Mitochondrial yolk.
c. Nucleolus	i. Nucleolar extrusions. ii. Secondary nuclei.
d. Cytoplasm	i. Cytoplasmic yolk.

*

EXPLANATION OF PLATES

(All the figures have been drawn with the help of a camera lucida under Leitz Microscope).

Preparations:—

Cedria paradoxa.	Figures 1-2	Ayoma, toned. Flemming without acetic acid and Champy, acid fuchsin.
Rhogas plecopterae.	4-5	Mann-Kopsch, turpentine.
	6—9	Flemming without acetic acid and Champy, acid fuchsin.
Dioctes gardneri.	10—12	Mann-Kopsch, turpentine.
	13-14	Champy, acid fuchsin.
Encospilus sp.	15-16	Mann-Kopsch, turpentine.
	17-18	Champy, acid fuchsin.
Trichomma nigricans.	19-20	Mann-Kopsch, turpentine.
	21-22	Champy, acid fuchsin.
	23	Centrifuged electrically, Champy, acid fuchsin.
	24	Intra-vita examination:— neutral red, Janus green B and 2% osmic acid. (all shown in one figure).

Magnifications:—

Figures	17, 18, 11 ..	X 40
	16, 20—23 . .	X 70
	24, 15, ...	X 80
	2, 7, 8, 9 ...	X 90
	10, 12—14, 19 ..	X 400
	3, 4, 5, 6 ...	X 700
	1, 6 ...	X 900

Explanations:—

PLATE I.

Cedria paradoxa.	Figures 1	An advanced young oocyte; peripheral vesicular Golgi bodies.
	2	An advanced oocyte; distinct and bigger vesicular bodies are present mostly at the periphery; crescents are seen; Golgi bodies in the act of fission; grey coloured cytoplasmic yolk bodies.
	3	An advanced oocyte showing activity of oocyte nucleolus; secondary nuclei formed from the extruded nucleoli; yolk bodies, granular and swollen mitochondria, infiltration, follicular secondary nuclei.
Rhogas plecopterae.	4	An young oocyte; Golgi bodies in the juxtanuclear position; grey granular bodies are mitochondria.
	5	An advanced oocyte with one nurse cell at the mouth of the oocyte as if it is being eaten up by the oocyte; vesicular Golgi bodies, inclusions are passing from nurse cell to oocyte.
	6	An young oocyte with juxtanuclear position of mitochondria.
	7	Portion of an advanced oocyte showing mitochondrial yolk bodies, swollen and filamentous mitochondria.
	8	Portion of an oocyte showing yolk formation; infiltration of mitochondria.
	9	Oocyte being crushed by the follicle cells—a case of atresia.
Dioctes gardneri.	10	An young oocyte with advanced juxtanuclear stage; Golgi fat vesicles seen.

11 Two oocytes in different stages of development; peripheral distribution of vesicular Golgi bodies; Golgi fat vesicles present.

12 An advanced oocyte with a direct connection with the oocyte chamber; the inflowing cytoplasm from the nurse chamber has thrown the inclusions of the oocyte to the periphery; vesicular Golgi bodies and Golgi fat vesicles are seen.

13 An young oocyte with a perinuclear arrangement of the granular mitochondia and some swollen mitochondria, perinuclear concentration of mitochondria in the nurse cells is seen.

14 An advanced oocyte with a direct passage between the nurse chamber and oocyte chamber; inflowing cytoplasm from nurse chamber has pushed the inclusions of the oocyte to the periphery; granular mitochondria and secondary nuclei at the periphery; infiltration, nurse cells in a degenerating stage.

PLATE II.

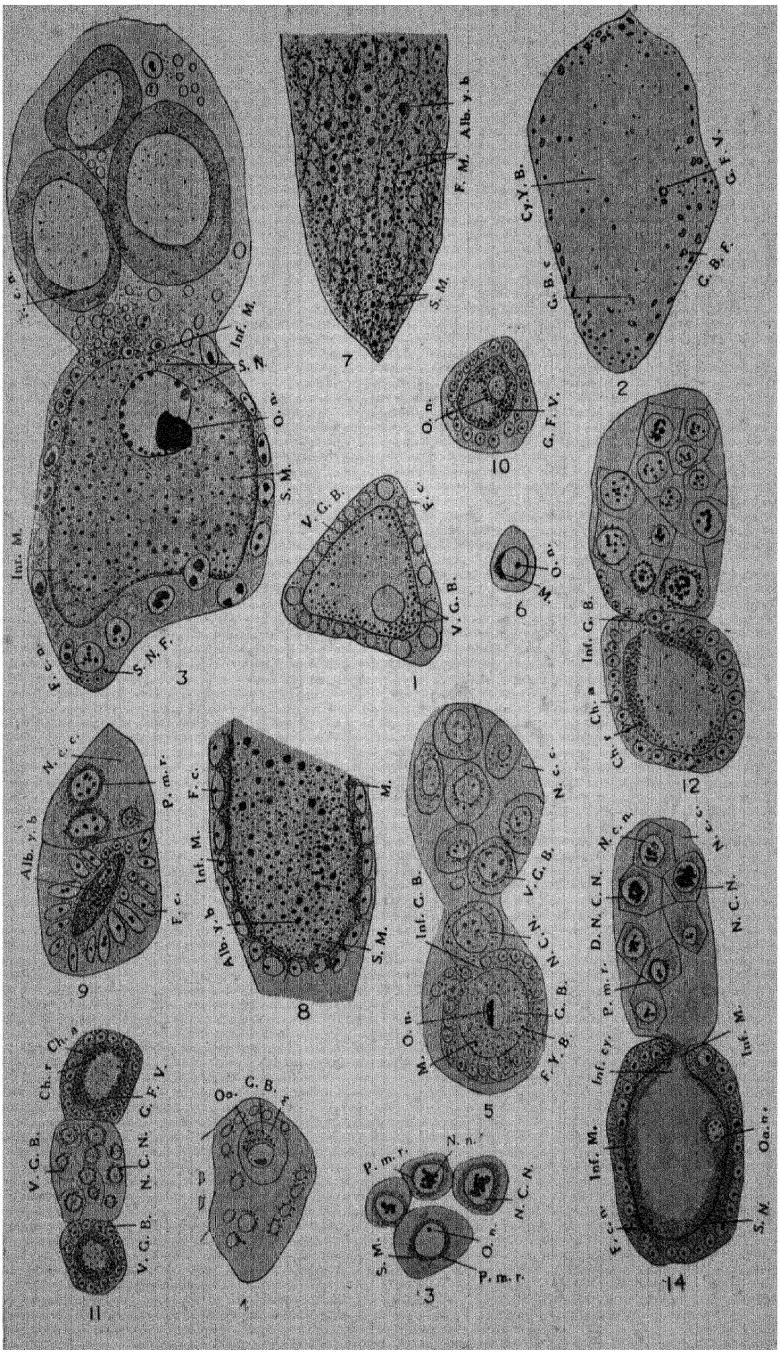
Enicospilus sp.

15 Portion of an ovariole containing oocytes in various stages of development.

16 An advanced oocyte showing a direct passage between the two chambers; vesicular Golgi bodies, Golgi fat vacuoles with chromophilic rim.

17 An advanced oocyte showing a direct continuity between the two chambers; inflowing cytoplasm; darkening of follicle cells; some degenerating nurse cells.

PLATE I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O. a.
S. N. F.
F. c. a.
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V. G. B.
F. c.
1
O. n.
G. F. V.
10
Cy. V. B.
G. F. V.
G. B. c.
C. B. F.
2
F. M.
Alb. y. b
S. M.
7
O. a.
6
Inf. G. B.
Ch. a
Ch. r
12
N. c. c.
Alb. y. b
P. m. r.
F. c.
9
Inf. M.
F. c.
Alb. y. b
S. M.
8
N. c. c.
V. G. B.
Inf. G. B.
N. C. N.
O. n.
C. B.
F. V. B.
5
N. c. c.
N. C. N.
D. N. C. N.
P. m. r.
Inf. M.
Oa. n.
S. M.
14
Ch. a
Ch. r
G. F. V.
V. G. B.
N. C. N.
V. G. B.
11
Oa.
G. B.
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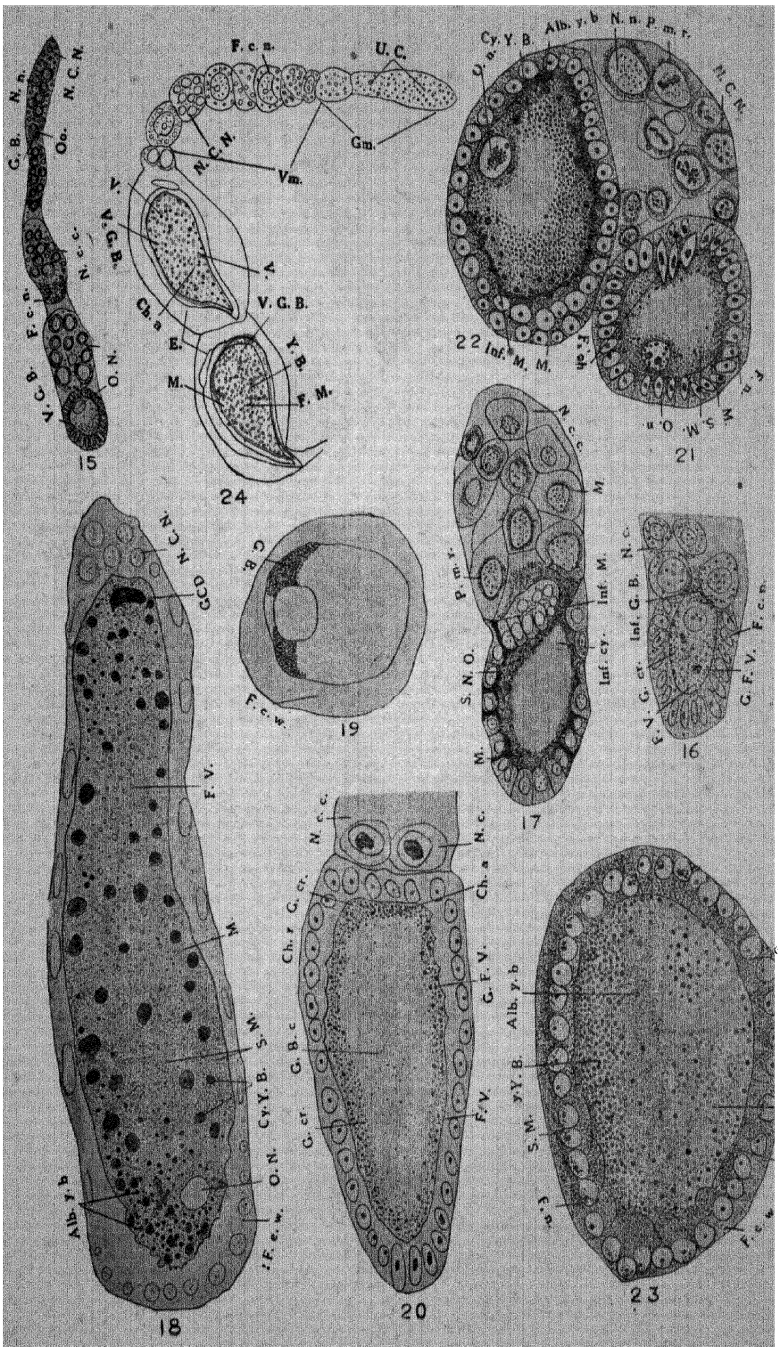
O. n.
P. m. r.
13

PLATE II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Gm.
Vm.
V.
V. G. B.
A.
Ch. a
E.
M.
V. G. B.
V. B.
F. M.
24
G. B.
N. n.
N. C. N.
Oe.
N. c. c.
F. c. a.
V. G. B.
O. N.
15
Cy. Y. B.
Alb. y. b
N. n.
P. m. r.
N. C. N.
22
Inf. M.
M.
F. ab.
F. a.
O. n.
S. M.
M.
21
N. c. c.
M.
P. m. r.
S. N. O.
Inf. M.
Inf. cy.
M.
17
N. c.
Inf. G. B.
F. V. G. cr.
G. F. V.
F. c. n.
16
G. B.
F. c. w.
19
N. C. N.
C. C. D.
F. V.
M.
S. M.
Cy. Y. B.
O. N.
Alb. y. b
F. c. w.
18
N. c. c.
N. c.
Ch. a
Ch. p
G. cr.
G. F. V.
G. B. c
F. V.
G. cr.
20
Alb. y. b
S. M.
Y. B.
23

18 A mature egg containing granular mitochondria, swollen mitochondria, mitochondrial yolk bodies and probably cytoplasmic yolk bodies; germ cell determinant.

Trichomma nigricans.

19 An young oocyte showing bipolar stage of Golgi bodies.

20 An advanced oocyte with vesicular Golgi bodies and Golgi fat vacuoles from which fat has dissolved out with turpentine.

21 An advanced oocyte showing granular and swollen mitochondria towards the periphery; three follicle cells have elongated and entering into the oocyte to eat it up; it is a case of atresis.

22 An advanced oocyte with granular swollen mitochondria; mitochondrial yolk bodies, infiltration, cytoplasmic yolk bodies, the later yellowish.

23 An advanced electrically centrifuge egg showing that the accumulation of mitochondria and cytoplasmic yolk bodies at the centrifugal pole; complete separation has not taken place according to the specific gravity of the inclusions.

24 Intra-vitam observations in the ovariole showing 'vacuome', Golgi vesicles, granular and filamentous mitochondria, yolk bodies, duplex nature of Golgi—all shown in one figure.

LETTERING

Alb.y.b	Albuminous yolk body.
Ch.r.	Chromophilic rim.
Ch.a.	Chromophobic area.
Cy.c.	Cytoplasmic connection.
Cy.f.	Cytoplasmic flow.
Cy.Y.B.	Cytoplasmic yolk body.
D.N.C.N.	Degenerating nurse cell nucleus.
Df.n.c.n.	Differentiating nurse cell nucleus.
E.f.	Egg follicle.
E.	Mature eggs.
F.ch.	Follicle cells migrating into the oocyte chamber.
F.c.	Follicle cells.
F.n.	Follicle cell nucleolus.
F.c.n.	Follicle cell nucleoli.
F.Y.B.	Fatty yolk body.
F.V.	Fat vacuoles.
F.M.	Filamentous mitochondria.
F.e.w.	Follicular epithelium wall.
F.C.N.	Follicle cell nucleus.
Gm.	Germarium.
G.B.	Golgi body.
G.B.c.	Golgi bodies in chains.
G.cr.	Golgi crescents.
G.C.D.	Germ Cell Determinant.
G.B.F.	Golgi bodies in fission.
G.F.V.	Golgi fat vacuoles.
Inf.G.B.	Infiltering Golgi bodies.
Inf.cy.	Inflowing cytoplasm.
Inf.M.	Infiltration of mitochondria.
M.	Mitochondria.

N.c.c.	Nurse cell chamber.
N.C.N.	Nurse cell nucleus.
N.C.	Nurse cell.
N.c.n.	Nurse cell nucleoli.
N.n.	Nurse cell nucleolus.
O.N.	Oocyte nucleus.
O.n.	Oocyte nucleolus.
Oo.n.	Oocyte nucleoli.
Oo.	Oocyte.
O.Dev.	Oocyte in different stages of development
P.m.r.	Perinuclear mitochondrial ring.
Pr.y.	Peripheral yolk bodies.
S M.	Swollen mitochondria.
S.N.	Secondary nuclei.
S.N.N.	Nucleolus of Secondary nuclei
S.N.n.	Nucleoli of secondary nuclei.
S.N.F.	Follicle cell secondary nuclei.
S.N.O.	Oocyte secondary nuclei.
T.n.w.	Thick nuclear wall.
U.c.	Undifferentiated mass of cells.
V.	Vacuome.
V.G.B.	Vesicular Golgi body.
Vc.	Vacuoles.
Vm.	Vitellerium.
Y.B.	Yolk bodies.

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STUDIES ON THE SEXUAL CYCLE IN THE LIZARD, HEMIDACTYLUS FLAVIVIRIDIS (RUPPEL).

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CONTENTS

CHAPTER 1.

INTRODUCTION.

The phenomenon of " heat " which, in lower animals, is defined as a periodic condition of sexual activity characterised by willingness to mate, as contrasted to the non-mating intervals, has, for a long time, been of great interest to the physiologists, the embryologists, the gynecologists, the obstetricians, and others concerned with the process of reproduction. The ovarian cycle, in only a few mammals and much less in lower vertebrates, has been studied by taking out ovaries from females killed at different periods. It has also been shown that the influence of various environmental changes often causes modifications in the reproductive activities of animals, e.g:—changes in temperature, humidity, food, etc. These factors often play a rôle in determining whether the spring or the autumn would be the common breeding season of the year. Even among the lower vertebrates, (viz., birds, reptiles, amphibians and fishes) that show a periodical expression of their sexual activity, it has been shown that the animals also display a definite correlation of their energy with certain seasons of the year such as the spring and the autumn which are usually the most favourable breeding times.

The remarkable progress which has been made in our knowledge of reproductive processes of mammals such as the guinea-pig, rat, mouse, opossum, sow, cow and sheep and possibly two or three more in which the cycle has been well studied by the application of an exact method, has not been made in other forms of vertebrates. This is perhaps partly due to the nature of the sexual cycle and partly due to the failure of most other group of vertebrates to breed normally under laboratory conditions.

In the human female, it has been known for a very long time, that the peculiar forms of diet, starvation, environmental conditions, both physical and chemical as well as psychological, exert a profound influence on the phenomenon of ovulation. In women certain mental condition may suppress menstruation and starvation inhibits the ovarian cycle entirely.

From the study of the previous literature it will be found that the relationship between the outside conditions and the structural changes occurring in the ovary in animals lower than man and mammals was not properly understood nor has it been intensively studied.

During the past several years my efforts have been mainly directed to studying the series of growth stages of the ovary and the testis of the lizard *Hemidactylus flaviviridis* in the breeding season. The common Indian gecko, *H. flaviviridis* is one of our widely distributed species found on the walls of every bungalow in the United Provinces. Its sexual cycle and breeding habits are practically unknown. Very few seasonal studies upon the reptilian gonads have so far been made.

My interest has been primarily directed to the study of the gonads of the lizard *H. flaviviridis* in order to determine whether there are seasonal variations in the volume and character of the cells and tissue of the gonads and if so to determine the relationship of testicular cycle with the ovarian cycle. If the interstitial tissue of the testes is supposed to control the sex characters and activity, there should be a definite correlation between the changes in this tissue and the appearance and disappearance of the breeding season With this possibility in mind, studies have been made of the seasonal relationship of the interstitial cells and the increase in volume of the testes and the ovaries; also of the behaviour of follicular cells of the ovary and of such of their other structural

changes as are involved within the discharged follicle after ovulation—the corpus luteum etc.

I undertook, further, to investigate the variations in the testes and the ovaries and to correlate these with the effects of temperature, weather and barometric pressure on the gonads in the hope that some conclusion could be drawn from the data observed as a result of the study on the sexual cycle in this lizard. It has not been possible to investigate the physical, chemical and psychological effects on the sexual activities of this reptile as has been done in the case of birds and mammals by Rowen (1925), but attempts have been made to determine if the influence of climatic changes such as temperature, humidity, etc., causes any modifications in its reproductive activities. The main object of this paper is to furnish as far as possible a complete account of the variations that have been observed in the testes and ovaries during the different seasons of the year with special reference to the changes in the discharged follicle (corpus luteum) of the ovary. The literature on the seasonal studies upon reptilian gonad is very scanty. Mingazzini (1893) and Lucien (1903) have given accounts of the corpora lutea in certain reptiles. Mazzetti (1911) has shown the interstitial cells to be abundant in the testes of a hibernating snake. They have been shown to be present in *Testudo orbicularis* by Pelligrini (1925). Frankenberger (1922), Reiss (1923), and Pelligrini (1925) have demonstrated the occurrence of the interstitial cells in various species of *Lacerta*. Blount (1929) has studied the same in the testes of the American lizard commonly called the horned toad:—*Phrynosoma solare*. A complete review of the work on seasonal modifications in the testes of vertebrates has been made by Oslund (1928). The above review emphasises the fact that the function of the interstitial cells and the source of testicular hormones are extremely problematical.

I wish to express here my sincere thanks to Dr. D. R. Bhattacharya, under whose direction this work was undertaken, for advice and kind criticisms. My thanks are due to him for suggesting this problem and for affording all laboratory facilities. The work has been carried on in the department of Zoology, University of Allahabad.

I desire to express my thanks to Mr. R. P. Rai for his valuable help in the preparation of the photographs and diagrams used in illustrating this paper.

CHAPTER 2.

MATERIAL AND TECHNIQUE.

The material upon which this investigation is based was collected during the years 1930 and 1931. The lizard *H. flaviviridis* is one of the commonest house geckos whose natural history, geographical distribution, food habits and lurking places are very completely known. (Gadow, 1920 and Boulenger 1890). It is a harmless animal and not at all difficult to capture.

It was chosen because of its several advantages for this type of work. In the first place it is easily collected throughout the year and secondly it has one definite breeding season each year. The gonads of this lizard are small and can be sectioned entire, thus avoiding cutting into parts. The testes and ovaries do not show any regional differentiation but respond to cyclic changes as a whole.

There are two species of the genus *Hemidactylus* found in Allahabad and surrounding regions—*H. flaviviridis Ruppel* and *H. gleadovi Murray*. But observations have been very carefully restricted to the one species *H. flavivirdis Ruppel*. All specimens of *H. flaviviridis* were secured at Allahabad and thus any variation due to climatic differences have been avoided.

The lizards were captured from the house walls and an attempt was made to keep a few in the laboratory. But as it was found difficult to feed the animal in captivity, it was killed and the gonads fixed in various fixatives soon after dissection. None was kept in captivity, all were living under natural conditions in this locality when killed.

Body measurements of adult mature lizards were taken and immature, deformed or otherwise abnormal animals were very scrupulously rejected. The average length of

the adult male was 6-7.5 cm. without the tail, and 1-1.3 cm. in width, while the female averaged 6.5—7.3 cm. in length and 1.5 cm. in width.

At regular intervals the animals were killed throughout the year. During the breeding season several male and female animals were killed almost every day, in other months of the year the intervals did not exceed more than two or three days. All specimens were either bled or decapitated and the gonads were quickly taken out and fixed in variety of fixatives. In the majority of cases the gonads were preserved with their ducts. Seasonal changes in the ducts of the gonads have not yet been studied and no attempt is made in this paper to correlate the changes in the ovary with the structural changes in the oviduct. Records of observed copulation numbered only half a dozen.

Size and volume measurements were recorded for all gonads. The number of ova in the ovary was also counted and never exceeded six. The measurements for length and diameter were taken with calipers to the nearest half mm. The volume of the testes and the ovaries after fixation was determined by displacement of water in a measuring glass. Careful records were kept of all data regarding each lizard and the meteorological observations were also noted for the day. The laboratory temperature was also recorded. The gonads after having been quickly taken out were placed in sufficient volume of fixing fluid in separate numbered tubes with dates and data written on the label.

The following fixatives were used:—Bouin's picroformol acetic fluid (Prof. Hill and Prof. Gatenby's formula was employed in the preparation of Bouin's fluid) was used as a fixing agent for the greater part, occasionally using Muller's formalin bichromate or only 5 per cent. neutral formalin, or Da Fano, or sometimes Champy-Kolatchev and Gerhardt fixatives.

The tissues were left in the fixing agent from 24 hours to 48 hours; then washed in 50 per cent alcohol for Bouin solution. They were then dehydrated in the usual way, cleaned in xylol, and embedded in paraffin. The gonads were cut after the usual microtechnical procedure, mounted, stained and studied. A thickness of six micra was found to be thin enough. Heidenhain's iron alum haematoxylin and Delafield's haematoxylin were the stains most frequently used.

The following injection experiment was performed with no obvious results:—The empty follicle after the discharge of the ovum (corpus luteum) was removed from the ovary and teased out and ground in absolute alcohol and injected in a gravid female. No change was observed either in the tubal eggs or in the ovaries. The eggs were laid in the scheduled time.

The collection of gonads in my possession gives a more complete series than any found in the literature previously recorded. Almost every stage is represented in the collection and all were in excellent state of fixation. The table gives the number of lizards dissected each time with dates. (Appendix).

Materials used with collection dates are as follows:—

Months.	Male.	Female.	Months.	Male.	Female.
January.	3.	8.	July.	8.	7.
February.	5.	8.	August.	8.	8.
March.	16.	18.	September.	11.	6.
April.	7.	27.	October.	6.	5.
May.	8.	16.	November.	11.	5.
June.	5.	12.	December.	4.	2.
Total.	44.	89.		48.	33.

Grand total 92 Males. 122 Females. (Please see Appendix)

CHAPTER 3.

PREVIOUS LITERATURE

The testes and the ovaries of teleosts as well as those of amphibians, birds and mammals have been studied at different seasons of the year by a number of investigators. Our knowledge of the morphology of the adult lacertilian testes and ovaries is very limited as the seasonal cycle of the gonads in the adult reptiles has been scarcely examined.

Most of the previous accounts on the subject deal with the amphibians and the mammals. Until recently, the cyclic changes of the gonads in animals which mate only at wide intervals did not attract the attention of investigators. Studies have been undertaken mostly to ascertain their maximum development and to establish a relation of the production of internal secretion of the testes and the ovaries with the periods of sexual cycle. The results are very varying and in many cases contradictory, which will be apparent by a study of the previous literature, a summary of which is given below.

Testes cycle

Leydig (1850) accurately described the interstitial cells of the testes of vertebrates. The tissue was named after him as Leydig's gland. He came to the conclusion that the interstitial cells of the testes influence the secondary sexual characters of the males of vertebrate animals. Many investigators, e.g., Champy and others, have since sought to determine the validity of Leydig's view. They have shown that an undoubted increase in the size of testes is correlated with the height of the sexual cycle.

Fishes—In fishes the seasonal variation in the spermary of the perch has been studied by Turner (1919). The testes showed marked regional differentiation.

Amphibians—Several investigators have published

accounts of the cyclic changes in the testis of anurans. Friedmann (1898) found the development of interstitial cells running parallel with the progress of spermatogenesis in frogs; *Rana fusca*, *Rana viridis*, *Hyla arboria* and in the toad *Bufo vulgaris*. According to him, when spermatogenesis has practically ceased, the interstitial cells tend to become reduced to their minimal size and number. On the other hand, when spermatogenesis is at its height and free spermatozoa are most numerous, the cells are of maximal size.

Mazzetti (1911), confirms these observations of Friedmann with regard to the frogs *Rana fusca* and *Rana viridis*.

The work of Champy (1908) indicates that the maximum development of the interstitial tissue in the amphibians follows the breeding season. Champy (1913), found in another species of frog *Rana esculenta* that the maximal development of the interstitial cells occurs when the phenomenon of spermatogenesis is at its lowest and vice-versa. The same conclusion is reached by Humphrey (1921) for *Necturus*. This condition appears to differ greatly from that reported by Friedmann and Mazzetti in the three species of frogs studied by them.

While Champy concludes that the interstitial cells in anuran testes have nothing to do with the sex characters, Aron (1924), however, found a definite relationship between the secondary sexual characters and the development of intertubular tissue of the tests both for *Rana temporaria* and *Rana esculenta*. Champy also states that in *Rana temporaria* the interstitial cells develop only after mating and disappear entirely during the progress of spermatogenesis. He has found in the toad *Bufo vulgaris* a condition similar to that in *Rana esculenta* and quite the opposite of that described by Friedmann.

Humphrey (1921) who has made a very comprehensive study of the interstitial cells of the urodele testes gives

explanation of the varied results of previous investigations on cyclic changes of the testes in closely related members of the same class of animals (Amphibians). He says, "that the different animals should differ in details of metabolism is inevitable. A degenerative change, for example, may be slightly delayed in one species and accierated in another. Such a change, then, considered as a force capable of affecting interstitial cell development, is differently applied in two closely related animals and may lead to difference in their interstitial cell development."

Reptiles.—No observations on the seasonal changes in reptiles have been made save the statement of Mazzetti (1911) that interstitial cells were abundant in the testes of hibernating snakes. Blount (1929), has shown that this was true of the horned toad *Phrynosoma solare.* Reiss (1923) has studied the secondary sexual characters of certain male lizards.

Birds.—In birds, Shattock and Seligmann (1914) report the condition of interstitial cells in the testes of wild ducks and in the fowl. The interstitial cells are smallest during the period of winter months but in the spring they undergo rapid development and persist in this greatly developed condition fully two months after the spermatogenetic cycle has ended.

The complete study of a member of this class was made by Stieve (1919) who worked on the testes of European jackdaw *Colaeus monedula.* He found that the interstitial tissue appeared more prominent in the testes in the months following the breeding season. There was no actual increase in the bulk of the interstitial cells. At the April mating time in this bird, the fluctuation in the size of the testes is 418 times the volume of the minimum size which occurs in January. Disselhorst (1908) gives the increase in testes weight in *Fringilla* at about 300 times that of the resting. In 1896, Gadow described testes volume changes in the house sparrow.

Watson (1919), found that in the green finch *Ligurnus chloris*, the testes were smallest with greatest number of interstitial cells during the inactive period and largest and most active during the month of May when the interstitial cells were fewest.

Rowan (1925) working on the sexual cycle in the North American snow-bird *Junco hyemalis* found that these birds migrate only when their testes are in a state of flux at the height of sexual activity.

Among others who have made special studies on the sexual cycle in male birds the following may be mentioned:—Boring (1912), Reeves (1915), Pézard (1918), Nonidez (1920-24), Benoit (1923), Bissonnette (1930).

Their conclusions will be considered more fully in the chapter on general discussion.

Mammals—Hansemann (1895) is the earliest worker in this field of study and has reported that in the rodents e.g. marmot the number of interstitial cells in the testes paralleled the size of the testes during the period of sexual activity and that at the time of spermatogenetic inactivity of the hibernation period during the winter season, the testes were in an apparently infantile condition with no interstitial cells. In the following spring when the active life was resumed and when spermatogenesis was more active they were greatly increased in number. Rasmussen (1917) substantiated the observations of Hansemann.

Ganfini (1903) on the other hand, declared that the interstitial cells of the marmot during hibernation were fully as numerous as during the active period.

Regaud (1904) and Lecallion (1909) worked on the testes of the mole *Talpa europa* and found that the increase in the size of testes was accompanied by increase in the number of interstitial cells during the approach to maximal spermatogenesis. Tandler and Gross (1911), working on the same material found that the interstitial cells

were at their greatest development after the breeding season. They found that spermatogenesis went on during the autumn and winter months, during which time the interstitial cells were at their minimal size and number. After the spermatozoa left the testes in March the interstitial cells increased greatly in size and number. With the increase in spermatogenesis in the early autumn, a reduction in size and number of interstitial cells occurred.

Marshall (1911), however, reports the greatest interstitial cell development during the breeding season in the hedge-hog *Erinaceus europaeus*. He has found no spermatogenesis during the winter but rapid enlargement of the testes in the spring due to the growth of the interstitial tissue. Regressive changes begin in October and the cells become so reduced in quantity as to bring the tubules into contact. Moore (1932) has made a comprehensive review of his own numerous publications on the subject as well as on those of other works in the field of the biology of testes.

It would appear from the literature cited above that a considerable variation occurs in the relation between the growth of the interstitial cells and the spermatogenetic activity in the testes of mammals. The works on these are contradictory. There is the same lack of uniformity in the birds and the amphibians. Very little work has been done on the reptiles.

Ovarian Cycle

The earlier works on the female reproductive cycle were mainly directed to the study of the corpus luteum in mammals, since the function of this important structure was considered to be the regulating of the commencement of sexual periodicity. The changes undergone by the ruptured follicle have also been studied in certain lower ver-

tebrates by Italian workers such as Malpighi (1689), Mingazzini (1893), Giacomini (1896) and others. As far back as the seventeenth century the corpus luteum was recognised and accurately described by Malpighi (1689) and given its name by the same writer.

Cyclostomes and Fishes—Giacomini (1896) has investigated the subject in birds, amphibians and more particularly in the elasmobranch fishes. Wallace (1903) gives a somewhat similar account of the spent follicles in the fishes—*Zoarces and Spinax*. In these fishes the author has found pronounced enlargement of the follicular cells with thecal ingrowth arranged in a radial manner. On the other hand, Buhler (1902) who investigated the ovaries of cyclostomes and certain teleosteans, was unable to find any hypertrophy of the wall of the spent follicle. Cunningham (1897) also writing on the teleosteans arrived at the same results as Buhler.

Amphibia and Reptiles—In the amphibians and reptiles very few contributions have been recorded so far. Giacomini (1896) has investigated the amphibians. He has found in the frog, however, that the cells of the discharged egg follicles persist for some time after ovulation without any marked change while in reptiles they hypertrophy to some extent. Lucien (1903) has described corpora lutea in the viviparous lizard *Anguis*. Similar structures in reptiles have also been observed by Mingazzini (1893) who believes them to be identical with the mammalian corpora lutea. Flynn has observed and described a placental structure in the lizard *Tiliqua*.

Birds—Giacomini (1896) has studied the corpora lutea in birds. Boring and Pearl (1917-18) have studied the genesis of interstitial cells in the female reproductive organs of the chicken and have described a corpus luteum in the ovary of the adult hen which they consider to be very much the same as that found in the ovary of sow. Fell (1925), in a series of papers on the histological studies of

the gonads of the fowl has definitely proved that a true corpus luteum does not occur in the ovary of the fowl.

Mammals.—The earlier papers written on the corpus luteum of the ovary of mammals largely dealt with the origin of luteal cells. They enquired whether these cells were of connective tissue origin according to Von Baer (1827) or were epithelial in nature as advocated by Bischoff (1842).

In 1834, Owen described and figured the copora lutea in the left ovary of an *Ornithorhynchus*. In the ovary of other mammals, Jan Coste (1847) has found that there is an effusion of blood into the ruptured follicle which is subsequently followed by histological changes in the follicle resulting in a corpus luteum. When the follicle contains no blood it is filled with a whitish coagulum. The hemorrhage may vary in amount or may even be wanting altogether as found by Foster (1879).

The function of the corpus luteum was suggested for the first time by Beard (1897) and it has withstood criticisms. On theoretical grounds, he attributed to the corpus luteum the function of regulating the onset of sexual activity and of preventing ovulation during the pregnancy. Beard's views were subsequently expressed by Prenant (1898) and Saunders (1902) but lacked a basis of facts and therefore were disregarded.

From 1903 to 1918 most of the papers published contain reports of experimental studies undertaken to prove that the corpus luteum is a gland of internal secretion. Marshall (1922) in his classical book on the Physiology of Reproduction has carefully reviewed the early literature on the subject of corpus luteum.

Born (1881) was the first person who properly understood the corpus luteum of pregnancy as a gland with internal secretion which in all probablity dominated the sexual cycle in mammals. A great deal of experimental work supports this theory.

Fraenkel (1903) tested experimentally Born's theory and was the first to recognise a relationship between the nutrition of the embryo and the secretion of the corpus luteum.

Leo Loeb (1906 and 1910) found that the corpus luteum secretes a substance which sensitizes the uterine wall at the time when the ovum fixes itself to the uterine mucosa.

Van der Stricht (1912) has very carefully studied the corpus luteum of the bat *Vespertelleo nuctula*. His conclusions are given fully in the chapter on discussion.

Corner (1915) has published a complete description of the corpus luteum of the domestic pig. He has attempted to give an account of the minute cytology of the luteal cells.

In 1917 Stockard and Papanicolaou discovered a new technique for the study of reproductive cycle in guinea pig by the method of microscopic examination of the smear of the vaginal contents. Long and Evans (1922) made a thorough study of the cyclic changes in albino rat using vaginal-smear method. This is regarded as a classical piece of work on the physiology of reproduction in rat. Next year (1923) Allen completed his study on the white mouse employing the same method.

The works of Professor J. P. Hill (1894—1926) and his collaborators e.g. Martin (1894), Sandes (1903), O'Donoghue (1913) and others, on the *Dasyurus*, and of Hartman (1923) on the *Opossum* are the most notable researches on the sexual cycle of marsupialia. Professors Hill and Gatenby (1926) have given a most complete description of the corpus luteum in monotremata—*Ornithorhynchus paradoxus*, which is perhaps the latest and most comprehensive work on the subject.

CHAPTER 4.

OBSERVATIONS

A. Seasonal Variation in the Testes.

(a) *Gross anatomical changes in the size of testes.*

The testes of the lizard *H. flaviviridis*, as of all other lizards are abdominal in position. Each is attached to the dorsal wall of the abdominal cavity by a fold of peritoneum—the mesorchium. The left testis is located slightly posterior to the right. Microscopic sections of the testis shows that it is composed of (1) outer tunica made up of the connective tissues, (2) convoluted seminiferous tubules, interspersed with, (3) interstitial cells. Blood vessels and lymphatics are numerous in the intertubular spaces. The testis in this animal strikingly resembles that of the mammal in the arrangement of the seminiferous tubules and presence of a definite epididymis.

The stages studied herein showed a remarkable change in the general appearance and the increase in size of the gonad—the cross section having a diameter ranging from 0.5 mm. to 6 mm. The interstitial cells, tightly packed between the convoluted tubes occurred in dense mass. Both the testes and the epididymes were soft and pliable. The colour varied from white to light yellow.

During the winter months—from November to February, Pl. 1 (Figs. 1—11) the testes were maximal in size with an average volume of 0.2—0.25 cc. and a length from 4—8 mm. and a diameter of 2.6 mm. The largest size attained by the testes at this time of the year (February), measured 8 mm. in length and 6 mm. in diameter. Early in the month of March, Pl. 2 (Figs. 12—14) they showed gradual decrease in size and more so in April

and May, Pl.2 (Figs. 15—19) when there was considerable sex activity. The regression of the gonad was very striking. The testes tubules enlarged very rapidly and stood out prominently under the tunic.

During the next three months—June, July and August, Pl·2 (Figs. 20—25) the testes steadily decreased in volume (minimum dimension in volume being 0.05 cc.) and in the later part of August they were considerably reduced in size. At this stage their minimum was reached, when they looked like a pair of small beads measuring not more than 1.5 mm. in length and 0.75 in breadth. This decline was most remarkable and its lowest limit was reached in August; Pl. 2 (Fig. 25). From September onwards Pl·2 (Figs. 26—32), there was a gradual increase in size and volume until October, and soon after the autumn resting stage the testes started increasing in size from the last week of October Pl·2 (Fig. 32) and continued the whole of winter months, i.e., November to February, but during this period the increase was very slow. The yearly cycle is thus completed. The maximum average volume is 5 times the minimum average volume.

(b) *Microscopical changes.*

Changes in the growth of the size of the tubules of the testes and in the volume of the interstitial cells.

The tubules of the testes—The testes are enclosed by a fibrous connective tissue layer—the tunica albuginea. The microscopic examination of the sections of the testes reveals the fact that the increase in growth of the gonad during the winter months of November to February Pl. 3 (Figs. 33—38) is consequent upon growth of the seminiferous tubules. There is a great contrast between the testes of the month of August and those of the breeding season. This is due to the relative diameter of the tubules and the size of the intertubular spaces.

The seminiferous tubules in late autumn and winter are completely filled by dense cytoplasm without evidence of cell walls. The germinal epithelial cells in the interior of the tubules appear to be in a state of rest. During the early winter the tubules are small with an average diameter of about 60—80/U. As the winter season advances they begin to enlarge. This is obviously due to active spermatogenesis which steadily continues upto April. The tubules reach a maximum diameter of 300 /U. In the later part of April, Pl. 4 (Fig. 43), the tubules are full of spermatogenetic elements. The sperms with wavy tail filaments and nearing maturity are present by the end of March, Pl. 4 (Fig. 39) and are completely matured by the first week of April and freed into the lumen within the next week of that month Pl. 4 (Fig, 48). From this date onward the testes retain mature sperms until the second week of May, Pl. 4 (Fig. 44).

At the close of the breeding season towards the middle of May and early in June there is a sudden change in the nature of testicular tissue, Pl. 5 (Fig. 45). After the spermatozoa are discharged, the tubules, which are almost empty and reduced in size, are lined by a single layer of flattened epithelial cells Pl. 5 (Fig. 46). Further regression of the already flattened germ cells of the tubules marked by vacuolation of the cytoplasm sets in. The decrease in the diameter of the tubules is due to the folding of their walls.

November—Inside each tubule the germinal cells are situated with small nuclei which usually form a single row in section. The cells within the tubules appear more crowded and there are no spermatozoa within Pl. 3 (Figs. 33—35).

December—The tubules are approximately the same size and as a whole is not sufficiently changed from the condition found in the previous month Pl. 3 (Fig. 36).

January—The tunic of the tubule—tunica propria—is

more distinct. The cells within the tubules have nuclei which seem to be more scattered throughout. The outermost cunica albuginea is becoming more fibrous, Pl. 3 (Fig. 37).

February—The tunica propria is thinner and tubules appear more crowded together and intertubular spaces are smaller. Nuclei of the cells in the tubules are more tightly packed. Tunica albuginea appear much more fibrous and thinner. At a later date in the month of February, the tubules have a diameter as large as the diameter of those on February 1st. Pl. 3 (Fig. 38).

March—The tubules are slightly larger in diameter than a week previously viz. the last week of February, Pl. 4 (Fig. 39—42). The cells increased in size to $7 \times 9/U$ and steadily continued increasing. The cells in the tubules are much more numerous. In many of the tubules a lumen is apparent. Spermatogonia with larger nuclei are to be seen nearer the tunica propria and primary spermatocytes nearer the lumen. Towards the third week of March, Pl. 4 (Fig. 42) secondary spermatocytes are present. Cells of different stages arrange themselves in definite zones. The tunica propria and the tunica albuginea are unchanged.

April—The tubules have increased in diameter since the last sample of March, Pl. 4 (Fig. 43). The process of spermatogenesis has been going on and cell generations are easily discernable. Tunica albuginea is much thinner. Towards the end of April mature spermatozoa are found free in the lumen of most tubules.

May—The tubules are smaller in diameter and cell generations are fewer, otherwise conditions remain the same, Pl. 4. (Fig. 44). The regressive stages in the series start from the middle of May.

June—August—By the 5th of June, Pl. 5 (Fig. 45) the testes have undergone regression attaining a condition equivalent to that of March in the progressive cycle. In

all cases so far observed it is the later stages of spermatogenesis that disappear first from the tubules in regression. Correlated with these changes inside the tubules are inverse conditions regarding interstitial cells, Pl. 5 (Figs. 45—50).

September—October—The regression, that occurs in the summer months in the testes of the adult male lizard, steadily continues leading to the conditions closely approximating those of winter, Pl. 5 (Figs. 49—50). No evidence has been found that the animal has any second period of sexual activity during the autumn. The Interstitial cells—The cells lying in between the tubules are abundant when the testes are in a state of rest during the month of August, Pl. 5 (Figs. 47—48). It is in the spring (March) when the tubules of the testes show greatest activity, that the interstitial cells are in their minimum. The cells are polygonal in shape with rounded nuclei, the cytoplasm of which usually containing fat globules. The cells are without cell boundaries. The syncytial condition is a characteristic feature at the close of the breeding season. As the winter sets in, the interstitial cells gradually assume definite polyhedral outline with rounded muclei.

November—Interstitial cells with well stained nuclei and granular cytoplasm are present between the tubules. In late November there is no change in the intertubular elements, Pl. 3 (Figs. 33—35).

December—The interstitial cells of the testes as a whole are not sufficiently changed from the condition found in the previous months, Pl. 3 (Fig. 36).

January—The interstitial cells appear to fill up all the intertubular spaces, Pl. 3 (Fig. 37).

February—The intertubular cells are relatively fewer, Pl. 3 (Fig. 38).

March—The interstitial cells are still to be found but much more scarce, Pl. 4 (Figs. 39—42).

April—The interstitial cells are found with difficulty, Pl. 4 (Fig. 43).

May—The condition of the interstitial cells remains the same, Pl. 4 (Fig. 44).

June—July—The changes inside the tubules bring forth an inverse condition regarding the interstitial cells. The testes showing regression of tubules show the interstitial cells in large number, Pl. 5 (Figs. 45—46).

August—In this month the interstitial cells attain maximum development, while the tubules are shrunken and have no apparent lumen, Pl. 5 (Figs. 47—48).

September—October—The condition of the intertubular cells steadily declines as the tubules gradually show signs of functional activity, Pl. 5 (Figs. 49—50).

The above account of the testes corresponds in general with the findings of Courrier (1921) and Rasmussen (1917-18) for woodchuck, (Mammalia), Humphrey (1921) for Urodela (Amphibia) and Blount (1929) for horned toad (Lacertilia).

(B). Analysis of data of testes cycle and meteorological changes.

(a) *Relation to temperature and barometric pressure.*

The table (appendix) and the graph (Plate 6) show the changes in volume of the testes at different dates. The testes which are largest in November and December, slowly decrease in size till February and by the middle of March, are of smaller dimensions. The daily maximum, mean and minimum temperatures and the barometric pressure for the period is shown in the table and the graph (Plate 6). There appears to be no parallelism between the temperature fluctuation and testes size. The spermatogenetic activity does not appear to be controlled by temperature change, nor even much modified by it. The correlation is that the decrease in the testes size

starts just about 21st March and it continues to decrease with the general rise of temperature in April and May until the end of August when the size of the testes is at a minimum as has been noted above.

No evidence is found that barometric pressure has any appreciable influence on the changes in size of the testes in the lizard. It may be a factor in the case of birds that migrate to mountains to breed as has been described by Bissonnette (1930).

(b) *Relation of change in testes to humidity and rain.*

From the observations recorded here it may reasonably be inferred that the reproductive activities of *H. flaviviridis* fall within a definite period of the year. It does not start earlier than the first week of April and ends about the first week of June. This period of the year in these provinces is the hottest and driest period with practially no rainfall. The presence of moisture in the atmospere is nil. The sexual activity of the lizard evidently seems to be inversely related to the atmospheric humidity and rainfall. The graph shows that the testes undergo maximum regression during wet August, when maximum humidity conditions are obtained.

(C) Seasonal Variation in the Ovary

(a) *Microscopical examination of the ovary.*

The gross structure of the ovary was studied in order to note the seasonal variations as also to correlate its activity with that of the testis.

November—February—The ovary underwent seasonal variation in volume as did the testis, but with somewhat greater and more abrupt changes. During the winter months *i.e.*, from November to February, Pls. 7 and 8 (Figs. 57 —74) the ovaries were small whitish bodies with two or three egg follicles while the testes

during these months were biggest in size. The dimension of the ovary was 2·5 mm. × 3 mm. and the volume 0·08—0·15 cc. whilst its weight on an average was 8—10 mg.

March—During the earlier part of the month of March, Pl. 9 (Figs. 75—79) the activity of the ovary in the female lizard was shown by a slight increase in size of the egg follicles. A week or two later in the same month, Pl. 9 (Figs. 80—84) the ovaries showed considerable change over the normal winter condition, Pl. 9 (Figs. 85—88).

April—May—In the early part of the month of April, Pl. 10 (Figs. 89—93) there was a marked rise of volume reaching an average of 0.8—1 cc, about ten times of the winter condition, and the follicles stood out prominently on the surface and their diameter reached 5 mm. During the later part of the month of April, Pl. 10 (Figs. 94—102) and the first half of May, Pl. 11 (Figs. 103—106) there was a sudden increase in the dimensions of the ovaries and by the end of April they measured 11 × 7 mm. and the volume reached a maximum of 1 cc. The individual follicle measured 7—10 mm. The developing ova assumed a yellow colour and as they matured they became deep orange yellow, Pl. 11 (Fig. 107—115).

Each ovary before copulation grew ten times in volume and weight than what was during the winter and was made up of about 6—8 follicles, one of which reached enormous size. Only two oocytes, one from each ovary, usually attained full size at the same time—two being the usual number of eggs laid by the lizard at one season of the year. The increase in size of the particular egg follicle was due to an accumulation of yolk.

During the month of April there was considerable sexual activity and copulation usually occurred during the first two weeks of the month. A few females were caught alive immediately after mating and brought to the labora-

tory and kept alive until the laying of the egg. Upon copulation the ovary showed marked changes. This was apparent by a study of the ovaries of a female killed during copulation. In one instance eggs were found to have ruptured from the two largest sized follicles of the ovaries soon after mating. As a result of this process the largest egg of each ovary was freed and after ovulation the ruptured follicles showed a partial collapse. The greatest number of lizards ovulated during the last two weeks of April from 15th to 29th. Pl. 10 (Figs. 98—102). Almost all the females captured at this period showed tubal eggs, the position of which could be located from outside through the thin semi-transparent abdominal muscles. By the middle of May all signs of ovulation had disappeared.

Gravid female lizards secured in the months of April and early May contained, when the abdomen was opened, an egg in each oviduct. It took about 12—15 days for the eggs to descend down the oviducts. The time which the eggs took for their journey down the oviducts was ascertained by close observation of the few females that were captured soon after copulation.

The discharged follicles left clear marks on the ovaries. The two ovaries were at once reduced considerably in size—so much so that they resembled the winter resting ovaries. After extrusion of the egg, the follicle, though much reduced in size, was visible as an oval structure having an aperture, Pl. 16 (Fig. 152) It remained as such until the egg was laid. Afterwards it degenerated rapidly and disappeared about the end of June.

The egg was fertilised in the oviduct, it descended down the oviduct and acquired a shell which was formed from the secretion of the shell gland of the oviduct. The egg laying period commenced about the fourth week of May (25th—31st) and lasted until the end of June.

June—October—The ovaries underwent rapid regression from June, Pl. 12 (Figs. 116—122) onwards and sunk Pl. 12 (Figs. 123—128) and Pl. 13 (Figs. 129—139) to resting stage, Pl. 7 (Figs. 51—57), i.e., from October to January till March Pl. 9 (Figs. 75—88), when their activity commenced again. So far as the size of the ovary and the testis was concerned it might be stated that when the ovary attained the minimum size the testis had the maximum.

(b) *Sexual behaviour and breeding habits.*

If one observes a female during the month of March, it will be seen that it always escapes if approached by a male, but at the beginning of April its behaviour will change and it will engage in a certain amount of sex play. The female lizard exhibits a most affectionate attitude towards the male, and if one examines the reproductive organs of the female in question at this time one finds them at the height of their sexual cyclic activity. The ovaries are found with ripe follicles about to rupture and the female is highly active and receptive to the male. It utters a peculiar sound and ere long copulation ensues.

In the case of some specimens, it is observed that the female lizards, isolated from males, lay eggs without mating. This suggests that in this lizard ovulation is spontaneous.

Females have been observed depositing their eggs in this period at secluded places such as behind book-shelves or upon the cornices of the walls. The eggs are perfectly spherical in shape. The shell is white and brittle and about 6 mm. in diameter. The size is not constant, however, for it increases as the embryo grows. Eggs kept under observation in the laboratory hatch within three weeks after laying.

(c) *Microscopic changes in the ovary.*

The ovary of the lizard is mainly composed of a few (3—5) egg follicles. A stroma of fibrous tissue does not appear to exist. (Plates 14 and 15).

Egg follicle—The egg follicles of the ovary clearly show a variation in size and form during the breeding season. During the non-breeding season, in December and January, Pl. 14 (Figs. 140-141) the follicles are so much reduced that they could hardly be identified. With the approach of the breeding season the ovary gradually increases in size. By the second week of March Pl. 14 (Fig. 143) the growing ovaries have assumed a beady appearance and the eggs look like small pearls. As soon as the yolk has been deposited which begins in April, Pl. 14 (Fig. 144) the spherules take on a pale yellow colour which gradually deepens. At this time there seems to be rather sudden growth of the ovary and the increase of the egg follicle is also remarkably rapid. By the middle of April and the beginning of May, Pl. 14 (Figs. 144—145) the egg-follicles are of mature size and measure in the ovary from 7-8 mm. in diameter. From June onwards Pl 15 (Figs. 146—151) the ovary declines rapidly to the winter resting condition.

During the early growth stages of the oocyte the follicular epithelium remains relatively thin. It consists of a single layer of cubical or polygonal cells arranged irregularly and enlarges when the oocyte is advancing in size. It forms layers of about 3-4 cells thick of which the innermost comprise very small cells. There are well marked cell membranes which delimit the follicular epithelial cells. Between the follicular epithelium and the theca interna lies a thin homogeneous matrix. The theca is uneven in thickness. Outside the theca interna is a well-marked layer of connective tissue—the theca externa.

Follicular atresia and the interstitial cells of the

ovary—A few instances of degeneration of the ovarian ovum and subsequent invasion of the same by the cells of follicle have been met with. Such follicular atresia is occassionally found after the breakdown of the ovum within the follicle. Fig. 169 (A. B.) shows an atretic follicle. The ovum is retained within the follicle and does not loose its circular shape. The cells of the follicular epithelium that break into the cytoplasm of the oocyte are smaller in size and the connective tissue of the theca takes no part in the process. Degeneration is entirely brought about by the follicle cells which multiply and eat up the cytoplasm and the yolk of the ovum which becomes filled with them.

Ovarian interestitial cells seem to be extremely variable and uncertain elements in the lizard. The cells that are found in the interspaces of the follicle are more of fibrous nature than glandular.

Histological changes in the discharged follicle.

The discharged follicles are situated one in each ovary, each having an opening through which the oocyte escaped. The opening is bounded by a folded rim and it leads into the follicular cavity as illustrated in figures (152—159) Plate 16. The figures represent low power view of a group of ovaries with ruptured follicles. They were all accompanied with eggs in the oviduct.

After ovulation the discharged follicle shows a collapse of its walls due to the loss of the contained ovum and of the follicular fluid. Pl. 16 (Figs. 152—159). The character of the follicular epithelial cells does not change all at once Pl. 16 (Figs. 160-161). The walls of the ruptured follicle grow steadily and consist of 5—7 layers of cells derived apparently from the follicular epithelium which becomes thickened by multiplication of its cells. The cells are fairly large with rounded nuclei and tend to grow into the empty follicle, the centre of which is occu-

pied by a coagulum which is continued to the surface of the ovary where the opening of the follicle occurred, (Fig. 162). Here it forms a plug. In the next stage, Pl. 17. (Fig. 163) the cells increase gradually in size and also in number and there is a tendency to fill up the empty capsule. Though filled up by cells only partially, the centre is usually occupied by the coagulum Pl. 17 (Figs. 162—168).

Further proliferation of cells occurs at the subsequent stage and the corpus luteum which is now 10—12 cell-layer in thickness does not appear to be a vascular structure as is generally found in the monotremata and other mammals. Sections through the ruptured follicles of fairly advanced stage of development will show much hypertrophy of follicular epithelial cells Pl. 17 (Figs. 165—168). They are very much unlike the large glandular luteal cells of the mammalian ovary. There is no indication of strands of connective tissue from theca interna branching out among the growing cells and there is no appreciable increase in size of the discharged follicle. It is very difficult to say that the follicular cells have, if at all, transformed into luteal tissue of the type that has been usually found in the monotreme ovary (Hill and Gatenby 1926) and in the ovary of other mammals described by Marshall (1904), Corner (1915) and others.

In its highest form of development the corpus luteum is essentially a mammalian structure, but it is particularly well developed in the monotremata which differ from the higher mammals in being oviparous. A corpus luteum in its fully formed structure such as found in the *Ornithorhynchus* is not usually formed in the lacertilian ovary. In the amphibia, the cells of the egg follicles only persist for some time after ovulation while in the lizard they definitely hypertrophy to some extent, but does not seem to transform into glandular cells.

In the corpus luteum of monotremata observed by

Profs. Hill and Gatenby (1926) three distinct periods have been distinguished :—

(1) A period of intra-uterine development.

(2) A period of incubation, and

(3) A period of lactation.

In the history of the lizard's sexual cycle, it has been found however, that the last two periods are altogether omitted. Only the first period viz. the intratubular period is present and obviously governed by the hypertrophied epithelial cells of the ruptured follicle. This structure is at its maximum development Pl.17 (Fig.165) when the eggs are located in the middle of the oviducts; but it is short-lived. Its life is only for about 15—20 days—the time which the eggs generally take for their journey down the oviduct. When the eggs are about to be laid the corpus luteum begins to show signs of atrophy. Cases of degeneration of tubal eggs were noticed side by side with the unhealthy state of the ovary and the ruptured follicle

The discharged follicle gradually becomes reduced in size until it disappears totally from the ovary by the end of June leaving only a trace of scar tissue and the oval area (Fig. 159).

Da Fano preparations of the lizard ovary (Figs. 160-161) with discharged follicle reveal clearly the cells in the interior of the follicle. They are provided with Golgi bodies which are minute granules stained black by silver.

Lucein has described the corpora lutea in the lizard *Anguis*. It is viviparous in so far as the young ones are fully developed, and burst the transparent soft yellowish eggs immediately after these are laid. Since the period of intra-uterine development is much too prolonged in *Anguis*, the hypertrophy of the follicular epithelium will be naturally greater and the structure will persist and remain functional for a longer period of time than what

is found in the *Hemidactylus*. The actual condition in viviparous lizard could not be studied; since a viviparous lizard was not available in these provinces, the observation therefore was limited to only the study of an oviparous one.

(D) Analysis of the data of ovarian cycle and the meteorological changes.

(a) *Relation of changes in the ovary to the variations in temperature and barometric pressure.*

The females of *H. flaviviridis* are not ready for functioning sexually earlier than middle of March and their activity reaches the maximum sometimes in the first week of May. As shown in the graph (Plate 13A) there is no relation between the variation of temperature and the size of the ovary. Like the testis, the activity of the ovary does not appear to be modified by the temperature fluctuation.

No general trend or marked tendencies in barometric pressure were noted during this period which could be responsible for the change recorded for the ovary of the lizard. Barometric pressure appears to be fairly constant during the sexual cycle, ranging from 29.4 to 29.8 mm. Hg. (Plate 13A).

(b) *Relation of ovarian change to humidity and rainfall.*

The mating begins in the middle of March, reaches its maximum in May and ends in June. The egg laying period starts from the middle of June. During these periods, however, the atmosphereic humidity and rainfall are at the lowest. The animals are sexually active at the time when the atmosphere is hot and dry and sluggish when it is cold and wet. This is presumably due to their being cold-blooded. The graph (Plate 13A) shows

that the change in volume of the ovary and the rainfall recorded at Allahabad on different dates. It may be gathered from the data that greater the humidity the smaller is the ovarian dimension and volume. Humidity therefore affects the ovarian growth in size and volume in inverse ratio as is the case in the testes.

*

CHAPTER 5.

DISCUSSION.

(a) *On the interstitial cells of the testes.*

It is certain that the gonads of many vertebrates furnish an internal secretion. This is shown by such evidences as the effects of castration e.g. experiments of Steinach (1918 and 1920), and those of Moore (1919 and 1932); in transplanting gonads according to the experiments of Nussbaum (1906), and of Steinach (1918 and 1920); by the works of Goodale (1916) in castrating ducks; and by Morgan's (1920) work on the common fowl.

The secretory function of interstitial cells of the testes is denied by Kingsbury (1914), Humphrey (1921) and Rasmussen (1918). The denial of such function for the cells is based on the irregularity of their occurrence and their seasonal variation in amphibians and reptiles, as in certain cases they reach their maximum size after the breeding season is over i.e., after the period of sexual activity.

Humphrey also denies the assumption that the secretion of the interstitial cells of Leydig is responsible for the development of the secondary sex character or sexual instincts or both. The mass of evidence supporting such an assumption is indirect and frequently conflicting when several forms of vertebrates are concerned.

Fishes—In studying the seasonal variation in the spermary of Perch, Turner (1919) has observed great regional differentiation which causes external constrictions and enlargements of the gonad. Observations have been made by Courrier (1921) on interstitial cells in the testes of the fish—sea-stickleback: *Gusterosteus aculeatus.*

He concludes that the interstitial cells produce internal secretion upon which the fish assumes the feature characteristic of "heat."

Amphibians—Champy (1908) has studied the interstitial cells in the testes of *Rana esculenta*. They undergo a measure of involution in July when the spermatogenesis is at its maximum. The involution of the interstitial cells of the testes results in the formation of the nutritive substance which sustains the previously formed spermatozoa. The author thinks that there is probably some internal secretion which is suggested by the disposition of the interstitial cells around the blood vessels. Champy (1913) finds in newts, salamanders and *Axolotl* a glandular tissue localised in the testes and appearing long after the sperms are expelled and disappearing at the height of spermatogenesis. This localised testicular tissue may be compared to a testicular secretory organ. The secretion has no direct effect on the secondary sex character but may have some other sex influences.

Aron (1924) states that the interstitial cells are localised, at certain seasons, in definite areas of the testes of *Rana esculenta*. In some species of urodeles regional differentiation is great, and even causes external constrictions and enlargements similar to those found in the teleosteans (Turner 1919).

Some authors: Mazzetti (1911), Rasmussen (1917), Humphrey (1921), claim that the interstitial cells revert to a connective tissue cell type as the breeding season approaches. Mazzetti says that the interstitial cells in the frog develop from the connective tissue and can be observed in all the stages through which they pass. He has also studied the testicular interstitial cells in various vertebrate types, and finds that they arise from a transformation of the connective tissue cells, and that they do not seem to have any importance in connection with the development of the secondary sex characters

which latter are determined by the re-absorption of the seminal fluid.

Reptiles—In the testes of the lizard *H. flaviviridis*, there is no such localisation of the interstitial cells as in the urodeles and teleosts and the interstitial tissue occurs in between the seminiferous tubules more or less uniformly distributed, though it exhibits seasonal variations. In this lizard I have not been able to observe any indication of a transformation of interstitial cells to connective tissue cell type. Blount (1929) has also found that there is no transition of interstitial cells to connective tissue cells in *Phrynosoma solare*. In the lizard *H. flaviviridis* an increase in the size of the testes subsequent to the breeding season has been observed. In the winter season, however, the increase in the size of the testes is due to the enlargement of the tubules. The interstitial cells were found to be present in small patches during the winter months, and in big lumps in the month of August when the breeding season was over and when the regression of the testes was at its maximum. An examination of the sections of the testis at this stage revealed that there was an enormous increase of the interstitial tissue resulting in the reduction of the size of the seminiferous tubules.

Birds—The conclusion arrived at by Boring (1917) after the examination of the interstitial cells of the testes of the young fowl is that there are no interstitial cells in the testes of the young fowl in the sense in which the term is technically used. Nor is there any evidence of an internal secretion of any kind formed by any cell of the interstitial tissue. Reeves (1915) calls attention to Boring's conclusions that there are no interstitial cells present at any time in the testes of chicken from 1 day to 12 months. He also calls attention to the work of J. des Cilleuls who found, on the other hand, interstitial cells from the 13th day onwards. Reeves examined the testes of cocks aged

5½, 9 and 18 months and found interstitial cells in all the stages examined.

Seligmann and Shattock (1914) have inquired into the correlation between seasonal changes in the testes and the plumage of the wild duck. The testes undergo a series of seasonal changes but the periods of non-activity and activity do not coincide with the two seasonal changes in the plumage. The condition of the interstitial tissue in these two periods is, according to the authors, that the cells reach their minimum during the winter, while in the spring they attain their maximum development and continue to remain as such for some time.

The investigations of Benoit (1923), Nonidez (1920), Pézard (1918-22) and Shattock and Seligmann (1914) have definitely established the fact that the condition of plumage and the development of the secondary sexual characters of the fowl are governed by the internal secretion of its gonads. But there exists diversity of opinion as to the particular cells which produce the secretion.

The experiments performed by Rowen (1929), Bissonnette (1930), Bissonnette and Chapnick (1930) and others, by manipulating the lighting conditions, have shown that the activity of the reproductive organs can be interrupted at will. There is a definite correlation between the migration of birds like junco and the change in their gonads.

Mammals—Hansemann (1895-96) states that in the woodchuck *Marmota monax* the interstitial cells are hardly represented at all during hibernation, whereas in the spring after the hibernation period is over, the interstitial cells are highly developed as in the wild boar, the cat etc., Ganfini (1903), and a few years later, Rasmussen (1917) find, like Hansemann, that the interstitial cells in the testes of the same rodent is reduced during hibernation but they point out that in reality there is no decrease in number of interstitial cells, the latter being only reduced in

size. Lecaillon (1909) studying the interstitial cells of moles' testes finds that these elements are very abundant in the non-active testes. Some of the cells degenerate and disappear which in part explain the reduction in volume of the testes. Marshall (1911) who has made a detailed investigation on hedge-hog, has found, on the other hand, that the interstitial cells increase in number during the period of reproductive activity i.e., the breeding season; but the majority of workers such as Tandler and Gross (1911) Humphrey (1921) Aron (1924) Champy (1924) have all found that they increase after the breeding season is over.

Tandler and Gross (1911), also working with the mole, found maximum testes size and spermatogenesis in March, with testes about three times their usual diameter and active spermatozoa in the tubules. This increase in the volume of the testes was found to be due to the increase in the germinal elements, and the interstitial cells were present only in small islands. With the approach of summer, the testes tubules diminished in size while the interstitial cells increased to a maximum. Winiwarter (1912) finds that the interstitial cells in the testes of man are variable elements. They exhibit fluctuations corresponding to the development and retrogression of the seminiferous tubules. The testicular interstitial tissue, according to Winiwarter presents close analogies with that of the ovary. In both cases the development is markedly cyclic.

The testis of the lizard *H. flaviviridis* behaves very much like that of the mammals. The interstitial cells decrease at the height of the breeding season, and increase at the close of it. They are in inverse ratio to the size of the testes and rate of spermatogenetic activity i.e., the maximum development of the testes tubules corresponds to the minimum of the interstitial cells. So far as the secretory functions of the interstitial cells of

the testes in this lizard are concerned, the writer cannot afford to be dogmatic, and will not attempt any answer to the statements of such authors as Kingsbury (1914), Humphry (1921), Rasmussen (1918), who deny such functions for these cells. From the study of literature, it seems, however, to be definitely established that the interstitial cells of the testes produce an internal secretion of the gonad which has profound influence in causing the condition of "heat,"—Bascom (1923), Ancel and Bouin (1909), Whitehead (1904) and Lipschutz (1919).

(b) *On the interstitial cells of the ovary.*

The term interstitial cell may be much more readily defined for the testis than for the ovary. Fraenkel (1905) and Schaeffer (1911) who studied a great number of different species concluded that these cells are not always present in the ovary. According to Winiwarter (1908) the interstitial tissue is present in the ovary of all species, and she explains the contradictory data as due to the researches being very incomplete. The interstitial cells are described as cells of a glandular nature.

Reptiles and Birds—Interstitial cells have not been recorded in the ovary of many lower vertebrate forms; perhaps they have not yet been investigated. Glandular interstitial cells were found by Goodale (1919) in relative abundance in the ovary of the fowl. The present investigation has shown that in the lizard *H. flaviviridis* the ovarian interstitial cells are extremely difficult to interpret, and the cells found in between the egg follicles appear to me to be more of the nature of fibrous than glandular tissue.

Mammals—By far the bulk of the work on interstitial cells in mammalian ovary has been purely descriptive, and based on examination of adult animals. The results are, however, of a very conflicting nature. In some mammals the interstitial cells in the ovary are fairly common and

well marked, but in the majority of mammals they are absent altogether. Aime (1907) published an account of his investigation on the interstitial cells of the ovary of some mammals. He finds that this tissue is a very inconstant structure and that it is therefore not essential organ comparable in importance to the corpus luteum. According to Aime the interstitial cells arise from the embryonic connective tissue.

In the rodents Dubreuil and Regaud (1908) have described in the ovary of the rabbit two distinct and extremely different types of interstitial glands. Firstly, there is a slightly developed gland which is very transparent owing to the abundance of lipoid bodies in the young cells. Secondly, there is a greatly developed gland the opacity and milky whiteness of which are due to abundance of fat. Many intermediate stages between these two extereme types also occur.

Regaud and Dubreuil (1909) also find that if a doe—rabbit is isolated, the interstitial gland diminishes greatly in winter, less in spring. Permanent cohabitation with a virile male determines an increase in the gland even during winter. Sexual activity in the spring and cohabitation are both associated with the increased development of the interstitial gland in the ovary. Variations in general nutrition do not account for the change in the gland. Loeb (1911) finds that the guinea pig's ovary has no interstitial gland like that of the rabbit. In the guinea pig during 'heat', spontaneous ovulation occurs not depending on preceding copulation. In the rabbit copulation is necessary as well as 'heat' to ensure ovulation.

In the insectivora e.g., mole (*Talpa europa*) and in some carnivora e.g., the bitch and the weasel, Popoff (1911) has found in the ovary numerous interstitial cells which are differentiated within the stroma at the expense of the theca interna. Kingsbury (1914) has studied the

interstitial cells in the cat ovary. According to him, they are modified stroma cells and hence of connective tissue origin. The cats have them in both embryonic and adult life. No evidence is found for regarding the interstitial cells as constituing an intra-ovarian gland in the cat. But in the dog, Marshall and Runchiman (1914) find that the occurrence of 'heat' in the bitch does not depend upon the presence of mature graafian follicle in the ovaries. It is equally evident that it is not dependant upon corpora lutea. The ovarian interstitial cells, according to the authors are possibly concerned in the oestrous process but cyclical changes in the condition of these cells have not so far been observed in the dog's ovary.

In the ungulates Bascom (1923) has found that the sow lacks the interstitial cells entirely, whilst in the cow they are absent through most of the embryonic life until near birth.

In the chiroptera O. Van der Stricht (1912) finds that there are no interstitial cells in the stroma of the ovary of the embryo bat *Vesperugo noctula*. They appear some days after birth at the expense of the connective tissue framework. Lipoid granulations and fatty globules appear in the cytoplasm of the intersititial cells just as in the corpus luteum. Interstitial glandular tissue was found in all the bats studied by Athias (1919). He also gave a very thorough description of the cytological character of this tissue. In *Vespertilionidae* it forms the greater part of the ovarian stroma and shows the attributes of glandular elements. In *Rhinolophidae* it is much less developed. It occurs in the ovaries of the embryo, young animals as well as in the adults. During pregnancy and lactation the interstitial tissue is at its maximum. In autumn it suffers considerable reduction and towards the middle of winter it begins to increase again. The interstitial cells occupy the whole extent of the cortical zone of the ovary.

In the bats according to the above authors the interstitial cells have glandular characters and they do not seem to have any thing to do with rut and ovulation but probably with the nutrition of the genital system and with the determination of the secondary sex characters.

Winiwarter (1908) has studied the interstitial tissue of ovary in human female and other mammals. According to her it occurs in all mammals including man and it appears periodically, as if in instalments and probably has a trophic rôle. Winiwarter (1908) defends the theory of trophic function while Athias (1919) and others uphold the endocrinal rôle. Salazar (1922) does not accord with the trophic theory. He has furnished facts from his observations on the ovary of rabbit which go to support the endocrinal theory. There is, however, no valid reason for speaking of an ovarian interstitial gland because the secretory function of the tissue is as yet unknown.

From what has been said it seems that the interstitial cells are variable elements of the ovary; the rabbit possesses them while the guinea pig does not, (Loeb 1911), the sow lacking them entirely, the cow lacking them through most of the embryonic life until near birth (Bascom 1923), the cat having them in both embryonic and adult life, (Kingsbury 1914). Interstitial cells were found in all bats some days after birth, (Athias 1919). In contrast such cells are present in the male testes throughout life (Van der Stricht 1912.) In the lizard ovary they are very uncertain structures and more or less fibrous.

(c) *On the histological changes of the follicular epithelium in the discharged follicle (corpus luteum) and in the atretic follicles.*

The cause that determines the sexual periodicity in the female has to be sought for in the ovary. It may be either the corpus luteum (Born 1881, Freenkel 1903, Re-

gaud et Dubreuil 1908 and Loeb 1911), or the liquor folliculi (Allen 1923) or the interstitial cells (Marshall and Runciman 1914).

The question of internal secretion in the ovary is in great confusion except for the recognised fact that the hormones are produced in the corpus luteum. Taniguchi (1916) finds in corpora lutea of the cow's ovary ten different ferments and almost the same in ovaries freed from corpora lutea.

From the time when the ductless glands first began to be known, there has been a tendency to regard them as physiologically more or less related to one another. During the recent years, a certain amount of information has been accumulated which renders it probable that there are true inter-relationships between the glands endowed with the power of internal secretion. Bell (1916) has given a connected account of his recent work and those of others which goes to show that the reproductive functions are directed and controlled by all the organs of internal secretion acting harmoniously together. According to him the influence of ovary on the general metabolism is related to and dependent upon its primary reproductive functions. The glands of internal secretion, such as the thyroids, the suprarenals and the pituitary, influence the development and subsequently preserve the integrity and activity of the genitalia. Other glands e.g., the thymus and the pineal, appear to prevent the sexual precocity. Contrariwise, insufficiency of the thyroids, the pituitary and the suprarenals may cause the cessation of the genital functions. Thus the individual metabolism and the reproductive metabolism are absolutely inter-dependant.

The histological changes in the tubes, uterus and vagina have now been linked on to the changes in the follicles and the corpora lutea, but the actual relationships between the follicle and the corpus luteum on the one hand

and the uterus on the other, are as yet largely unexplored.

Recent biochemical works on ovarian hormones and physiological experiments of ovarian cyclic activity, such as those performed by (1) Parkes (1928) by injection of œstrin isolated from placenta, liquor amnii, faetal membranes, and ovarian extract; (2) Smith and Engle (1927) by injection of anterior pituitary extracts; (3) Allen and Doisy (1923) by injection of liquor folliculi; (4) œstrous symptoms detected by Stockard and Papanicolaou (1917) by employing vaginal smear technique, (5) Parkes and Bellerby (1927) by using an acetone, precipitated ether extract of corpus luteum ground in anhydrous sodium sulphate, (6) Loeb (1910) by removal of both ovaries, (7) Knaus (1927) by removal of corpus luteum, are the studies outside the scope of the present discussion since very little experimental work has been performed except the injection of an alcoholic solution of the teased out empty follicle in the female lizard with no outstanding results.

From the previous literature the following data have been compiled as to the fate of the discharged follicle in the vertebrate groups for purposes of comparison with that of the spent follicles in the ovary of the lizard *H. flaviviridis*.

Fishes—Giacomini (1896) has investigated the elasmobranch fishes and describes an hypertrophy of the follicular epithelium consequent upon ovulation. The discharged follicle of the *Myliobatis* is described and figured as a glandular body in which the enlarged epithelium is penetrated by an extensive ingrowth of connective tissue and blood vessel. Wallace (1903) gives a somewhat similar account of the spent follicle in teleostean fish, *Zoarces*. Buhler (1902) and Cunnigham (1897) both working on the histology of the ovary in certain cyclostomes and marine teleostean fish *Coreoganus*, were unable to

find any overgrowth of the wall of the empty follicle. This is perhaps due to the fact that the elasmobranchs being viviparous showed the marked hypertrophy of the discharged follicle while the teleosteans being mostly oviparous showed little change in the follicle.

In some of the slides prepared in this laboratory by one of the research students who examined the ovaries of of pregnant *Scoliodons*, I have noticed a pronounced hypertrophy of the follicular epithelium; thus confirming the observation of Giacomini on *Myliobatis* ovary. Dr. B. T. Char, I am informed, has likewise worked out the corpus luteum in certain fishes. His observations which are still unpublished leave no doubt about the occurrence of corpus luteum in fishes.

Amphibians—Giacomini also investigated the amphibians in which the cells of the egg follicles persist as such for sometime without any noticeable change after ovulation. Duschak (1924) in *Rana temporaria* and *Rana fusca* found no hypertrophy of the coats of the follicle after the discharge of the ovum but Hett (1923) found enlargement of the granulosa in *Triton vulgaris*.

Reptiles—In the reptiles the follicle cells actually hypertrophy to some extent. Lucein (1903) has described corpora lutea in the reptile e.g. *Anguis* in which there is a simple hypertrophy of the follicular epithelium. It will be seen that owing to this lizard being viviparous in so far as the young ones are fully developed and burst out immediately after the eggs are laid, a pronounced hypertrophy and a corpus luteum lasting beyond the average period is naturally expected. The condition of the corpus luteum in viviparous reptiles other than *Anguis* is not definitely known. In the present communication a structure similar in appearance to the corpus luteum has been found in the lizard *H. flaviviridis* but it lasts for a very short time only, since this lizard is oviparous in habit. Mingazzini (1893) has also observed the same structure

in other reptiles and believes it to be identical with mammalian corpus luteum. Hett (1924) has reported a corpus luteum in *Lacerta agilis*, but no yellow pigment was observed.

Birds—Raymond Pearl and Alice Boring (1918) demonstrated the homology of the corpus luteum in the hen and in the cow. The course of its development in the hen is an abbreviation of that in the cow. It corresponds to the late involution stage of the cow corpus lutem since both contain a yellow fatty substance as shown by Sudan III, absolute alcohol and xylol reaction. According to these authors, in the hen, a corpus luteum is formed in both discharged and atretic follicles. Its origin is definitely from the theca interna.

Precisely similar cells, "luteal" to all appearance occurs in the stroma of the ovary of young hen and may be true interstitial cells according to Goodale (1919). It has been shown that the so-called "luteal" cells in the ovaries of the embryo of the fowl are always derived from the sex cord (Pfluger's) i.e. epithelial cells.

The statement of Pearl and Boring (1918), that in the atretic and in the discharged follicles of the hen these luteal cells multiply, migrate and ultimately obliterate the cavity and give rise to a glandular structure homologous with the cow corpus luteum, has been doubted by Hartman (1923), Hartman and Hamilton (1922) and Nonidez (1922).

Honor B. Fell (1925) has re-examined the ovary of the adult hen and concluded that the tissue which occupies the cavities of the atretic and discharged follicles is derived from the follicular epithelium and shows differences from the luteal cells described by Pearl and Boring (1918) who homologise the structure with the corpus luteum of the cow. There is no significant point or resemblance between the corpus luteum of a mammal and the tissue in the discharged follicle of the fowl.

According to Fell a true corpus luteum does not occur in the ovary of the fowl.

Raymond Pearl and Frank M. Surface (1914) find that the ovulation of an actively laying hen is immediately inhibited by the injection in proper dosage of the dessicated fat free substance of the corpus luteum of the cow. It has been shown by Loeb (1911) that one function of the well developed corpus luteum in the mammalian reproductive cycle is to inhibit ovulation. Its substance does the same in birds where there is nothing corresponding to the corpus luteum. The authors have also found that the active substance of the corpus luteum in producing the inhibition is inactivated by boiling.

Mammals—Bouin and Ancel (1909) distinguish two kinds of mammals in so far as the ovulation is concerned:—(1) Mammals with spontaneous ovulation and (2) mammals in which the ovulation is provoked by copulation The former have two kinds of corpus luteum, according as the ovulation is not or is followed by fertilisation. These two kinds are distinguished as the periodic corpora lutea of menstruation (corpora lutea spuria) and corpora lutea of pregnancy (corpora lutea vera). Mammals with non-spontaneous ovulation have only the second kind of corpus luteum—the gestative corpus luteum. To the first category the authors refer man, primates, dog, horse, cow and pig. To the second kind rabbit, guinea pig, mouse and cat. The ovaries of the first set have no interstitial gland. It may be said that the periodic corpus luteum corresponds to the intersititial gland which occurs in mammals of the second set.

Normally, ovulation comes shortly after mating but this time is extremely prolonged in the bat in which copulation is performed during the autumn while ovulation is postponed until the following spring (Van der Stricht 1912). Another example is the marsupial

Dasyurus in which the ovulation is delayed 4-8 days post coitum. (Hill and O'Donoghue (1913).

Monotremes—A corpus luteum occurs in the duck-mole, *Ornithorhynchus paradoxus* which has been very thoroughly investigated and described by Hill and Gatenby (1926). The authors are of opinion that on the 8th day there are blastocysts in the uteri varying somewhat in their degree of development from the primitive streak to the flat embryonal stage. They have a temporary attachment over the ant-embryonal pole and in those more advanced, the definitive ectoplacental attachment is being effected. During this period the corpus luteum is at its highest development. The luteal cells come from the follicular epithelium alone. In an interesting article, M. Lee Grade (1930) has extended the work of Profs. Hill and Gatenby on the ovary and corpus luteum of *Ornithorhynchus paradoxus*.

Various earlier authors e.g. Owen, (1834), Caldwell (1887), Sandes (1903), Marshall (1903—04) and O'Donoghue (1914) have testified to the existence of the corpus luteum in monotremes.

Marsupials—O'Donoghue (1914) has studied the corpus luteum in the marsupials. In these, the corpus luteum cells are derived exclusively from the cells of the membrana granulosa while the proliferation of the theca folliculi gives origin to the connective tissue of the corpus.

In the marsupial *Dasyurus*, according to Sandes (1903) the corpus luteum attains its full development at the time when the uterine blastocyst is 7 mm. in diameter. It remains in the same state for 7-8 weeks after the birth of the young. Then it begins to regress and by the time the young one is about 10 cm. long (some 4 months after birth), there remains no trace of the corpus luteum in the ovary which is found to be full of young ova beginning to grow in preparation for the next reproductive period. Hill and O'Donoghue (1913) look-

ed upon *Dasyurus* as unique in the long delay of ovalation after 4-8 days post coitum.

Hartman (1923) on the other hand finds that in the *Didelphys* the corpora lutea decline much more rapidly than that of *Dasyurus* and in 4 or 5 weeks they have all disappeared. According to Hill and Gatenby (1926) this remarkable difference in the time of regression of the corpus luteum is related to a possible difference in the sexual cycle of these two polyprotodont marsupials—*Dasyurus* being monoestrous and *Didelphys* polyoestrous.

In the ungulates and in the carnivores according to Corner (1915, 1919, 1923) there is recurrence at regular intervals of a condition of sexual excitement and impr gnation of the female if copulation takes place. In the domestic pig there is a three-day period of "heat" throughout the year. The sheep has, in autumn (or in some breeds in autumn and spring), a sequence of œstrous period. The dog is in 'heat' for a week or more but twice annually. Corner (1915), in the case of the corpus luteum of the pig, states that it reaches its full development on the 8th day when unattached blastocysts are present in the fallopian tubes. It persists in full vigour in the pregnant female and only undergoes regression about the time of parturition.

In many rodents the œstrous period is not conspicuous externally. According to Evans and Bishop (1923) the œstrous excitement in the rat is not due to corpora lutea but to ovarian structures which reach their height before the corpus luteum has come into being. Long and Evans (1922) observe in the white rat that the corpora lutea are fully formed or are in vigorous function 2-3 days after ovulation. Huber's (1915) findings also agree with those of Long and Evans when the fertilised eggs pass from the tubes to the uteri. Towards the end of pregnancy marked changes are observable. The corpora lutea undergo

diminution in size. They persist, however, throughout the period of lactation since the phase of cellular proliferation in the mammary gland is determined by the corpus luteum.

In the case of mouse, Sobotta (1906) states that the corpus luteum attains its definite structure 3 days after ovulation when the eggs are present in the fallopian tubes. A regression in the corpus luteum does not occur.

Fraenkel (1903) carried out an experiment with rabbit to determine the functions of corpus luteum during pregnancy as a gland of internal secretion. He cauterised corpora lutea in rabbit at various stages of pregnancy and noted that removal of corpora lutea during the first half of pregnancy led to a destruction of the embryo and abortion, and in the second half of the pregnancy the operation was without any effect. He concludes that the corpora lutea control the retention of the embryo in the early stage when the placenta is not properly developed. But this conclusion is not certain. The early removal of corpora lutea prevents the continuance of pregnancy in all species except the guinea pig. In this rodent the corpus luteum is not necessary to the continuance of pregnancy; at any rate the removal may not always be followed by the destruction of the fœtus. In the rabbit the result of removal of the corpus luteum was constant in causing termination of the pregnancy according to Fraenkel, while Niskoubina (1909) found that destruction of the corpora lutea in the rabbit was without any effect after 14th day of pregnancy. In view of these conflicting results it is impossible to come to any conclusion applicable to all mammals because the removal operation of the corpora lutea, however, was not considered specific. The exact cause of the sexual cycle may lie in some other part of the ovary or the whole ovary itself.

Bouin and Ancel (1909) have experimented with rab-

bits and have reached the following conclusions as to the influence of corpus luteum on the uterus and the mammary gland. The corpus luteum determines the structural transformation—such as hypertrophy of the mucous epithelium and richness of the blood supply that are observed normally in the uterus during the first part of the gestation. The implantation of the ova depends on the integrity of the corpora lutea. Continuing their studies on the rabbit the authors (1909) find evidence that there is a close correlation between the corpus luteum and the development of the mammary gland. It conditions the cellular multiplications which induce the development of the mammary gland during pregnancy.

In the rabbit, Niskoubina (1909) distinguishes a period of development of the corpus luteum extending over 4-5 days after coition. The glandular activity of the corpus luteum ceases abruptly towards the 15th day, that is about the middle of the gestation. Thereafter the corpus luteum begins to show signs of a progressive atrophy. Cohn (1903) states that the corpus luteum attains its maximum size after 8 days, whilst Marshall (1903—04) is of opinion that at that time the corpus luteum is very nearly fully formed.

Regaud and Dubreuil (1908) have made experiments, which they regard as convincing, that the corpora lutea do not condition rut. But Villemin (1908), whose results are criticised by Regaud and Dubreuil, maintains his previous conclusion that in the rabbit, as in other mammals, rut is determined by the internal secretion of the cells of the corpus luteum. O. Van der Stricht (1912) discovered that the ovulation in the bat is extremely prolonged. He studied the corpus luteum in a number of chiroptera. He insists on two clearly marked periods of secretion—one of serous secretion and the other of lipoid secretion. But he gives no cytological evidence for his conclusion. In the bat *Vespertillio noctula* he

distinguishes three phases in the history of the corpus luteum :—

(1) a phase of development extending from ovulation up to the fixing of the blastocyst (2) A phase of full functional activity (3) A phase of regression which begins a little before the birth of the fœtus.

In the sexual cycle of human female according to Falkiner (1933) the corpus luteum of menstruation has only a short period of activity—14 days more or less. Should pregnancy occur, there is no doubt that the corpus luteum will be given a new lease of life and will persist for many months as an active structure. When the duration of the life of the corpus luteum is much prolonged, it prevents new ovulation and calls forth growth processes of the mammary gland.

Fraenkel (1911) observed that menstruation failed to occur in women in whom corpora lutea had been removed by operation for some reason or other. Hartman (1923) has contended that there is an active substance originating outside the ovaries that causes the periodic bleeding or menstruation. He announces on experimental evidence that it is the anterior pituitary that elaborates the substance causing uterine bleeding.

Loeb (1906) found that profound changes go on in the uterus preparatory to the fixation of the ovum. The decidual tissue is produced by stimulus of the ovum acting upon the endometrium sensitized for pregnancy by the action of the corpus luteum. The corpus luteum secretes a substance which sensitizes the uterine wall at the time when the ovum fixes itself to the uterine mucosa.

In the human ovary Gatenby (1924) has found blood clot in the cavity of the discharged follicle, and according to him, the luteal cells are almost follicle-epithelial in origin, but certain of the theca cells are said to become very much like the glandular true luteal cells in appearance. These he distinguishes as para-luteal cells.

In women the period of regression, which begins in the later part of pregnancy and sometimes before child birth, takes a long time to complete as it has to control the functioning of the mammary gland and prevent ovulation.

From the above accounts it will be clear that in the history of the corpus luteum in the viviparous animals, a period of genesis followed by functional activity is usually attained by the time the blastocyst travels down the oviduct and its attachment to the uterine wall is being accomplished.

In the case of the sexual cycle of the oviparous lacertilian *H. flaviviridis* under examination, it will be found that during the first 8-12 days after ovulation the cells in the interior of the discharged follicle show a marked hypertrophy because they have to perform several important functions:—

(i) to control the proper nourishment, (ii) to ensure the safe conduct of the tubal eggs down the oviduct and (iii) to prevent a synchronous passage of the two large eggs through the cloaca. The fact that some cases of degeneration of the oviducal egg have been observed side by side with unhealthy state of the discharged follicle will evidently go to show that it performs no less important function. Compared with the *Ornithorhynchus* corpus luteum, the hypertrophied cells inside the empty follicle of the lizard ovary differ from those of the former in one fundamental respect, and that is: while the latter has no phase of full functional activity, there are three phases in the development of the corpus luteum of monotremes (Hill and Gatenby 1926). In the corpus luteum of the lizard there is but one phase which corresponds to the first phase, viz ; extending from ovulation upto the time of laying of the eggs, i.e. during the time the blastocysts remain in the oviduct. The second phase viz. the period of functional activity, which is most pronounced in the *Ornithorhynchus*, is altogether omitted in this lizard since

like the former there is no laying of the blastocyst either in a nest or hatching in a temporarily formed external pouch, also there is no functioning of the mammary gland as in the monotremes. The degenerative changes in the discharged follicle of the lizard set in rather early and by the time the egg is ready for laying the structure totally disappears from the ovary.

From the study of the lizard ovary it seems as if the corpus luteum, which is so commonly found in the ovaries of monotremes and other mammals, had its inception in the reptilian ancestor. In the eutheria the structure grew up and acquired functions in conformity with the physiological needs of the animals. In those viviparous reptiles e.g. *Tiliqua* in which a placental structure has been described (Flynn), whether the corpus luteum has a cycle similar to that found in the mammal, is a question which further research alone can answer.

Cytology of the cells of the discharged follicle.—The Golgi bodies stained black by silver have been seen in the cells of the interior of the discharged follicle of the lizard. The Golgi apparatus and mitochondria in the luteal cells have been studied by Requier (1910) in the cow, *Bos taurus*, Goormaghtigh (1929) in the dog, and Hill and Gatenby (1926) in the *Ornithorhynchus*.

Requier (1910) finds in the cow that the Golgi reticular apparatus, though varied in appearance, is usually evident in a perinuclear position. A network is not often visible. The Golgi bodies finally end in practically complete disappearance. Requier used the Golgi method which distorts the apparatus to a great extent.

In the corpus luteum of human ovulation according to Gatenby (1924) the Golgi apparatus of the lutein cells retains its eccentric juxtanuclear position in almost every cell. The spreading out and the circumnuclear situation of the Golgi apparatus seem to occur in the cow (Requier), the rat and other lower mammals. Lutein granules ac-

cording to Gatenby (1924) are formed from the mitochondria—since these granules are arranged like the mitochondria and all are equal in size.

Atretic Follicle.—In the ovary of the lizard *H. flaviviridis* the ovarian ova degenerate inside the follicle and subsequently the cytoplasm is attacked by the proleferated follicular epithelial cells which tend to occupy the oocyte. Dubuisson (1906) has stated that in the bird (sparrow), the follicle cells may multiply and act as phagocytes to the yolk. Sometimes the ovum becomes filled with them. A similar process is observed and described as occurring in the crocodiles by me (in part I). Perez (1903) also has recorded the phagocytic absorption of the ova by follicle cells in the ovary of the newt. Follicular atresia in mammalian ovary has been described by several previous workers a summary of which is given by Branca (1925). According to Popoff (1911) the atretic follicle is the outcome of the interstitial glands which develop in moles and weasels at the expense of the elements of the internal theca. In the dormouse, *Eliomys quercinus*, Winiwarter (1912) has observed that the part played by the connective tissue in forming the atresia during the embryonal stage progressively persists in the adult ovary. It is the penetration of the vasculo-connective tissue in follicular cells which brings forth the atresia. But Salazar (1924), who has investigated the ovary of rabbit, declares that the connective tissue has no atrifying property. In the lizard, however, atresia is brought about by the follicle cells.

(d) *On the relation of gonads to environmental variants.*

Among the environmental variants, nutrition is not indicated as a major factor for the fluctuation of the size of the gonads, for all the lizards caught at different times were well nourished. An attempt to analyse the stomach contents in order to find out the possible influence of

changes in type of food was made for some time but was given up later on, because it was found that the lizard is exclusively insectivorous in habit. It is well known that there are two sharply defined breeding seasons each year for the vertebrate animals (except man) other than those that hibernate during the autumn and winter. "The spring and autumn are the seasons appointed for the amours of birds and many species of fishes" (Spallanzani 1784). Reference to records of temperature, barometric pressure, humidity etc., shows that these bursts of reproductive activity always take place at time when there is a marked change of climate—the one in autumn shortly after a sudden change from great heat to cooler weather, the other in the early months of the year when the cool winter weather gives place to spring season.

With the cold blooded reptiles, however, autumn season is by far less favourable than the spring. In the case of the lizard *H. flaviviridis*, particularly, the rise in temperature decreases the size of the testes in the late April, while at the same time it increases the ovarian size. Fluctuation in temperature in the late spring does not retard the steady regression of the testes until the middle of August. During the winter months, however, the testis grows in size and its growth is inversely related to that of the ovary. Variations in temperature in the spring season act as one of the prime factors in spermatogenetic and ovarian activities. Later on the summer temperature has little effect on the breeding habits.

Barometric pressure changes did not show any marked controlling factor, for it was fairly constant; being steady between 29.4 and 29.8 mm. Hg. throughout the year.

(e) *General conclusion.*

In autumn and winter the ovaries of *H. flaviviridis* are extremely small in size. The testes on the other hand

are fairly big in size. The testes are yellowish in colour and the ovaries are minute and white.

In the spring, the ovaries increase slowly in size in early March, and somewhat more rapidly in the later part of the same month; very rapidly in April and first half of May. At this time the testes decrease to about 2-3times their winter size and are greyish white. The ovaries enlarge very much and are pink white containing 2-3 large follicles with equal number of small ones.

In the later half of May both the ovaries and testes show sudden and rapid decrease in size. The ovaries reach almost the winter condition in the later part of July and first half of August. The testes then start growing in size until their maximum is attained in the winter (December-January) months. *H. flaviviridis* breeds only once a year and no evidences were found to infer the existence of a second period of sexual activity during the year, for the results have been checked all through two summers—1930 and 1931. I have not been able to conduct any experiments with a view to ascertain the influence of variations in temperature and humidity on the sexual activity during the dormant season.

With regard to the behaviour of the interstitial cells in lower vertebrates, Benoit (1923) found them in the bird *Cambasson* very abundantly being fat-laden in the winter when spermatogenesis has ceased. He thinks that they become glandular in the spring and are certainly much fewer. Pahron and Pahron (1922) do not find these cells more glandular in the spring in Rouen geese. Many investigators believe that they may act as nutrient cells, e.g. Sainmont (1905), Tandler and Gross (1911). Others believe that they change in response to change in the size of the tubules, e.g. Whitehead (1904), Kuntz (1919), Humphrey (1921-25) and Oslund (1924). Oslund concludes that the parallelism between spermatogenesis and sexual activity is evident; while there is an

inverse relationship between sexual activity and interstitial cells.

That the interstitial cells in mammalian testes and ovaries exert a formative influence on sex character has been experimentally determined by Steinach (1920) and confirmed by Lipschutz (1922) and others. When Steinach implanted ovaries in early castrated males of rats and guinea pigs these males were feminised, the mammary glands were stimulated, milk was secreted, the growth of the penis was inhibited. Similarly female was masculinised. Lipschutz (1924) observed a penis-like growth of the clitoris. The action of the interstitial cells forming a puberty gland is specific.

It has also been shown by Goodale (1916) and Pezard (1918) that a young cock, castrated, keeps the characteristic plumage and grows spurs, but does not gain the normal combs and wattles. A young hen castrated gains masculine plumage and spurs.

The study of the interstitial cells in the lizard gonads, however, adds little or no evidence in favour of their interpretation as a gland of internal secretion. It is perhaps because of their non-glandular nature that there are no indications of the presence of contrasting secondary characters of sex in *H. flaviviridis*.

In the lizard sexual cycle, however, there is no apparent histological difference in the interstitial cells when found at the different dates. There is no sign of the cells becoming glandular even in the spring. My data appear to corroborate the conclusions of Benoit (1923) Tandler and Gross (1911) and Watson (1919) in regard to spring changes in birds. They also support the conclusions of Oslund (1928) in so far as the behaviours of the interstitial cells are concerned and agree with the findings of Blount (1929) in regard to the volume changes in testes of *Phrynosoma solare*.

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CHAPTER 6.

SUMMARY.

Gross morphological and histological studies have been made of a series of testes and ovaries of the lizard *H. flaviviridis* obatined at frequent intervals throughout two years.

The study has shown that a definite correlation exists between the interstitial cells of the testes and the appearance and disappearance of the breeding season and has also thrown some light on the behaviour of the follicular epithelial cells inside the discharged follicle (corpus luteum).

The Testes Cycle.

It has been shown that the testes varies in size. It is largest in winter months from November to January. It starts regression slowly through February. The change becomes more rapid and pronounced in March when a rapid decrease in cellular tubule contents occurs. Then in April and May it decreases very rapidly when the spermatogenetic activity reaches its full limit.

The total testes volume and the amount of the interstitial tissue in different seasons were taken into account. *H. flaviviridis* being cold blooded shows adaptivity of the gonads and the gonidial activities to variations in temperature. The interstitial tissue exhibits a definite cyclical phenomenon. When the testes were at their smallest size the interstitial cells are greatest in amount and they are fewest relatively when the activity of the germ cell is at its height. The changes in the interstitial cells are correlated with the increase in tubule length and breadth and the volume of the testes. Interstitial cells are in inverse ratio to the degree of spermatogenesis and they do

not give any indication of any secretory activity in their appearance at any time.

Regression in size of the testes takes place towards the end of May at the time of highest summer temperature; variation in barometric pressure is not indicated as conditioning regressive changes.

No evidence was found to indicate a second onset of spermatogenesis.

Ovarian Cycle.

All adult female lizards pass through a definite reproductive cycle, which begins in the first week of the month of April.

The time in which changes in the ovary occur, is coincident with the testicular changes. There is an exact correlation between the ovarian cycle and the time of ovulation with the testicular cycle. Each ovary shows enormous increase in size of one follicle during the breeding time in April.

The height of the breeding season occurs early in April. Copulation has been observed in April and during the first half of May. This is usually the time of ovulation. The two largest eggs generally get discharged after copulation, but ovulation may also occur in the absence of copulation.

The epithelium of the discharged follicles shows hypertrophy. The follicular epithelial cells undergo marked proliferation and form the corpus luteum which persists during the passage of the eggs down the oviduct. The corpus luteum begins to degenerate when the eggs are laid and ere long are reduced to mere scars. For the first time these structures have been found and described in the lizard *H. flaviviridis*.

CHAPTER 7.

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—— 1912.	(See testes cycle).

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CHAPTER 8.

EXPLANATION OF PLATES AND DESCRIPTION OF PHOTOGRAPHS AND FIGURES

A. *Testes cycle of H. flavviridis.*

Plate 1.—Photograph showing seasonal variations in the size of the testes.

Figs. 1—4 November X 2.

Figs. 5—6 December X 2.

Figs. 7—9 January X 2.

Figs.10—11 February X 2.

Plate 2.—Photograph showing seasonal variations in the size of the testes.

Figs. 12—14 March X 2.

Figs. 15—17 April X 2.

Figs. 18—19 May X 2.

Figs. 20—21 June X 2.

Figs. 22—23 July X 2.

Figs. 24—25 August X 2.

Figs. 26—28 September X 2.

Figs. 29—32 October X 2.

Plate 3.—Microphotograph of transverse section of the testes showing changes in the tubules and in the intertubular tissue.

Figs. 33—35 November X 60.

Fig. 36 December X 60.

Fig. 37. January X 60.

Fig. 38. February X 60.

Plate 4.—Microphotograph of transverse section of the testes showing changes in the tubules and in the intertubular tissue.

Figs. 39—42 March X 60.

Fig. 43. April X 60.

Fig. 44. May X 60.

Plate 5.—Microphotograph of transverse section of the testes showing changes in the tubules in the intertubular tissue.

Fig. 45. June X 60.

Fig. 46. July X 60.

Fig. 47. August X 60.

Fig. 48. August X 30.

Fig. 49. August X 60.

Fig. 50. September X 60.

Plate 5A.—Microphotograph of transverse section of the testes showing changes in the tubules and in the intertubular tissue.

Figs. 51—52 October X 60.

Plate 6.—Graph illustrating the seasonal cycle of the testes in relation to meteorological changes.

B. *Ovarian cycle of H. flaviviridis.*

Plate 7.—Photograph showing seasonal changes of the ovaries with oviducts.

Figs. 51—57 October X 2.

Figs. 58—61 November X 2.

Plate 8.—Photograph showing seasonal changes of the ovaries with oviducts.

Figs. 62—67 December X 2.

Figs. 68—71 January X 2.

Figs. 72—74 February X 2.

Plate 9.—Photograph showing seasonal changes of the ovaries with oviducts for the month of March.

Figs. 75—79 Beginning of March X 2.

Figs. 80—84 Middle of March X 2.

Figs. 85—88 End of March X 2. (with tubal eggs).

Plate 10.—Photograph showing seasonal changes of the ovaries with oviducts for the month of April.

Figs. 89—93 Beginning of April X 2.

Figs. 94—97 Middle of April X 2.

Figs. 98—102 End of April X 2. (with tubal eggs).

Plate 11.—Photograph showing seasonal changes of the ovaries with oviducts for the month of May.

Figs. 103—106 Beginning of May X 2. (with tubal eggs).

Figs. 107—110 Middle of May X 2.

Figs. 111—115 End of May X 2.

Plate 12.—Photograph showing seasonal changes of the ovaries with oviducts.

Figs. 116—122 June X 2.

Figs. 123—128 July X 2.

Plate 13.—Photograph showing seasonal changes of the ovaries with oviducts.

Figs. 129—136 August X 2.

Figs. 137—139 September X 2.

Plate 13A.—Graph illustrating the seasonal cycle of the ovaries in relation to meteorological changes.

Plate 14.—Outline of the T. S. of ovaries showing growth stages of egg follicles in different months.

Fig. 140 December X 10.

Fig. 141 January X 10.

Fig. 142 February X 10.

Fig. 143 March X 10.

Fig. 144 April X 10.

Fig. 145 May X 10.

Plate 15.—Outline of the T. S. of ovaries showing growth stages of egg follicles in different months.

Fig. 146 June X 10.

Fig. 147 July X 10.

Fig. 148 August X 10.

Fig. 149 September X 10.

Fig. 150 October X 10.

Fig. 151 November X 10.

Plate 16.—The Corpus luteum in the ovary.

Fig. 152 shows two large tubal eggs and the ovaries with corpora lutea. X 2.

Figs. 153—159 illustrate suciessive stages of the corpus luteum, at the centre of which there is an aperture through which the ovum escaped. X 2.

Figs. 160—161. Transverse sections of the corpus luteum showing proliferation of the follicle cells. X 60.

Plate 17.—Microphotograph of transverse section of the corpus luteum showing stages of its development.

Fig. 162—Early stage X 60.

Fig. 163—Slightly advanced stage showing hypertrophy of the follicle cells.

Fig. 164—Fairly advanced stage X 60.

Fig. 165—Advanced stage showing proliferation of the folliculer epithelium X 60.

Fig. 166—Regressive stage of the corpus luteum X 60.

Fig. 167—Further regressive stage of the corpus luteum X 60.

Plate 18.—Cells of the corpus luteum and atretic follicle.

Fig. 168—Structure of the hypertrophied follicle cells (luteal cells) drawn with the aid of camera lucida under oil immersion X 1000.

Fig. 169—Microphotograph of an atretic follicle X 60.

APPENDICES

SIZE AND VOLUME CHANGES OF THE OVARY.

Date of Killing.	Lizard Number.	Length of the lizard from Snout to Vent.	Size of Ovary L. xB	Volume of ovaries with oviducts.	Number of eggs in each ovary Right Left.
Jan. 3	1	6.5 cm.	4 x 3 mm.	0.1 cc.	4 eggs
Jan. 23	5	6 cm.	3.5 x 2.5 mm.	0.1 cc.	6 „
Feb. 21	8	6 cm.	2.5 x 3 mm.	0.1 cc.	5 „
Feb. 22	9	6.5 cm.	2 5 x 2.5 mm.	0.08 cc.	5 „
Feb. 26	11	6.5. cm.	3.5 x 3 mm.	0.1 cc.	5 „
Feb. 27	12	6.5 cm.	3.5 x 3 mm.	0.1 cc.	6-5 „
Feb. 28	13	6 cm.	3 x 2 mm (R)	0.1 cc.	
			4 x 3 mm (L)		6-2 „
March 6	14	7 cm.	6 x 3 mm (R)	0.125 cc.	
			5 x 2 mm (L)		5-4 „
March 8	15	7.5 cm.	4 x 3 mm (R)	0. 1 cc.	
			3.5x2 mm.(L)		5-4 „
March 9	16	6.5 c.m.	4 x 3 mm	0.1 cc.	3-4 „
March 10	17	6 cm.	3.5 x 2.5 mm	0.1 ec.	4-3 „
March 11	18	6. 5cm	4 3 x mm.	0 1 cc.	5-5 „
March 12	19	6 cm.	3 x 3 mm.	0.1 cc.	4-4 „
March 14	21	6 cm.	4.5 x 3.5 mm.	0.1 cc.	5-5 „
March 18	22	6. 75 cm.	5 x 4 mm	0.12 cc	6-6 „
March 19	23	6 cm.	5 x 4 mm.	0.2 cc.	5-5 „
March 20	24	7 75 cm.	6.5 x 5 mm,	0 3 cc.	4-5 „
March 21	25 A	6. 5 cm.	4 x 4 mm.	0.2 cc.	6-4 „
March 22	25 B.	7 cm.	6 x 8 mm.	0.2 cc.	6-5 „
March 23	25 C	6. 8 cm.	5 x 5 mm.	0.18 cc.	5-3 „
			(Ovulation has occured).	0.3 cc.	3-4 „
March 24	26	6 cm.	2 x 1 (ovulation)	(with tubal eggs)	(with corpus).

ENVIROMENTAL VARIANTS : TEMPERATURE BAROMTERIC PRESSURE ETC.

Maximum Temp.	Minimum Temp.	Mean Temp	Barometric Pressure.	Humidity (Saturation 100).	Rain.
73.1	47.6	60.3	29.7	80	0
71.8	41.6	56.7	29.8	74	0
90.2	54.6	72.4	29.7	71	0
89.8	57.6	73.7	29.7	71	0
94.0	60.6	77.3	29.6	58	0
87.0	56.8	71.9	29.6	30	0
82.8	59.6	71 2	29.6	30	0
86 8	56.6	71 7	29.6	32	0
92.0	60.6	76.3	29.6	51	0
98.3	63.6	80.9	29.6	36	0
91.8	57.1	74.4	29.7	32	0
93.3	60.9	76.9	29.6	30	0
95.5	58.6	77.0	29.5	26	0
97.8	59.6	78.7	29.6	41	0
99.1	61.8	80.4	29.7	28	0
102.0	70.1	86.0	29.4	31	0

SIZE AND VOLUME CHANGES OF THE OVARY.

Date of Killing.	Lizard Number.	Length of the lizard from Snout to Vent.	Size of Ovary L. XB.	Volume of ovaries with oviducts.	Number of eggs in each ovary Right Left
March 25	27	6 cm.	5X3 mm (ovulation)	0.8 cc. (with tubal eggs). 1 cc ,,	3-2 eggs. 2-2 ,,
March 27	28	6 cm	5X2 mm. (ovulation)	1 cc ,,	4-4 ,, (without Corpus).
April 1	29	6.6 cm.	5X2. 5mm. (ovulation)	1 cc ,,	5-6 ,, (with corpus)
April 3	30	7 cm.	7.5X5 mm. (observed copulation.)	1.5 cc."	3-4
April 4	31	7.4 cm.	4.5X2mm (ovulation)	1cc. .5 cc (left ovary only).	2-3 ,, (with corpus)
April 5	32	7.2 cm.	6X3mm.	1.3 cc (Both with tubal eggs).	Very Vascular 5-3 eggs (No corpus)
April 11	34	6.5 c.m.	5X3 mm (Ovulation)	0.8 cc (on tubal eggs).	4-4 eggs
April 12	35	6.5 cm.	6X7 mm.	0.9 cc.	5-5 eggs
April 13	36	6.5 cm.	5X3 mm. (with tubal eggs)	0.8 cc.	2-4 ,,
April 15	37	7.5 cm.	6X4 mm.	1 cc. (W.T.E.)	5-5 ,, with corpus.
April 20	38 A	7 cm.	5X3 mm.	1 cc.	4-4 ,, (Copulation).
April 20	38 B.	6.7 cm.	4X3 mm. (with tubal eggs).	O.5 cc (W T E)	
April 21	39	7 cm.	4X4 mm. (without tubal eggs).	0.02 cc.	6 eggs.

ENVRONMENTAL VARIANTS: TEMPERATURE BAROMETRIC PRESSUE ETC.

Maximum Temp.	Minimum Temp.	Mean Temp	Baro-metric Pressure.	Humidity (Saturation 100).	Rain.
102.5	66·1	84.3	29.5	41	0
92.9	67.4	80.1	29.7	46	0
88.8	65.6	77.2	29 6	56	0
99.0	69.6	84.3	29.5	40	0
102.0	72.6	87.3	29.5	26	0
102.9	73.8	87.9	29.6	63	0
98.8	70.6	84.7	29.6	22	0
106.8	79.8	93.3	29.4	24	0

SIZE AND VOLUME CHANGES OF THE OVARY

Date of Killing.	Lizard Number.	Length of the lizard from Snout to Vent.	Size of Ovary L. x B.	Volume of ovaries with oviducts.	Number of eggs in each ovary Right Left
April 22	40	6.6 cm.	4 x 4 mm (without tubal eggs)	0.02 cc.	4 eggs
April 23	40 A	6 cm.	5 x 3.5mm. (with tubal eggs).	0.01 cc.	6-7 ,,
April 24	41	6.5 cm.	5 x 4 mm.	1 cc (W.T.E.)	3-2 ,,
April 25	42	7 cm.	8 x 7 mm.	1 cc.	5-6 one big egg (Copulation)
April 28	44	6 cm.	8 x 7 mm.	0.8 cc.	6-7 one very big egg.
April 29	45	6.5 cm.	9 x 8 mm (with tubal eggs).	0.7 cc.	5-4 (Do).
April 30	46	6.5 cm.	7 x 5.5 mm. (no tubal eggs.)	2 cc (with tubal eggs).	3-2 eggs
April 30	47	7 cm.	11 x 7 mm.	0.5 cc	7-6 eggs.
May 1	48	6 cm.	5 x 3 mm. (no tubal eggs).	1 cc.	4-6 eggs.
May 5	49	6. 5 cm.	6 x 5 mm.	0.6 cc.	6-4 eggs. (eggs are laid).
May 6	49 A	6.5 cm.	4 x 3 mm. (with tubal eggs.).	1 cc. (W.T.E)	4-3 eggs.
May 9	50	6 cm.	3 x 2.5 mm. (no tubal eggs).	0.5 cc.	3-3 eggs.
May 13	51 A.	6 cm.	3 x 2 mm.	0.1 cc.	1-2 egg (tubal eggs are laid.
May 16	52	6 cm.	2 x 2 mm.	0.3 cc.	4-5 egg. 6 small eggs.
May 18	54	6 cm.	3 x 2 mm.	0.1 cc. (W.T.E.)	3 eggs with corpus luteun.
May 19	55	6 cm,	5 x 4 mm.	0.2 cc.	3 eggs.

ENVIRONMENTAL VARIANTS: TEMPERATURE BAROMETRIC PRESSURE ETC.

Maximum Temp.	Minimum Temp.	Mean Temp.	Barometric. Pressure.	Humidity (Saturation 100).	Rain.
104.8	76.0	90.4	29.3	14	0
107.8	75.2	91.5	29 4	28	0
106.8	75.8	01.3	29.5	14	0
108.2	74.8	91.5	29.4	16	0
110.8	82.6	96.7	29.4	40	0
106.8	77.8	92.3	29.5	47	0
104.5	82.1	93.3	29.3	43	0
101.8	79.0	90.4	29.5	35	0

SIZE AND VOLUME CHARGES OF THE OVARY.

Date of Killing	Lizard Number	Length of the lizard from Snout to Vent.	Size of Ovary L. x B.	Volume of ovaries with oviducts.	Number of eggs in each ovary Right Left.
May 20.	55 A.	8.3 cm. (largest specimen)	1.5 x 1mm.	0.05 cc.	4 eggs.
May 26.	56	7 cm.	6.5 x 5 mm.	0.3 cc.	3-4 ,,
May 27.	57	6.5 cm.	4 x 3 mm.	0.1 cc.	8-6 ,,
May 28.	58	7 cm.	1.5 x 1 mm.	0.1 cc.	4 ,,
May 29.	58 A	6 cm.	1 x 1 mm	0.05 cc.	4 ,,
May 30.	58 B	6.5. cm.	1 x 1 mm	0 04 cc.	Ovary reduced to a cord.
June 4.	59	7 cm.	0.8 x 0 5 mm,	0.25 cc.	3 eggs.
June 9.	60	7 cm.	0.2x0. 1 mm.	0.15 cc.	2-3 eggs extremely small eggs on the ovarian cord.
June 12.	61	7.75 cm.	0,2x0. 1 mm.	0.1 cc.	3 eggs.
June 13.	62	7 cm.	0 2x0. 1 mm.	0.1 cc.	3 eggs about invisible.
June 16.	63	7.75 cm.	0.15x0.08mm.	0.1 cc.	,,
June 19.	64	6.75 cm,	0.15x0. 1 mm.	0.1 cc.	,,
June 22.	65	7 cm.	0.2 x0. 1 mm.	0.1 cc.	4-5 eggs.
June 23.	66	6.7 cm.	0.1 x0.05mm.	0.06 cc.	2-3 eggs (beady.)
June 26.	68	7 cm.	0.25x0. 1mm.	0.06 cc.	3-4 eggs.
June 27.	69	7 cm.	0.7 x0.5 mm.	0.06 cc.	4-5 ,,
July 1.	71	6.5 cm.	1 x 0.5 mm.	0.1 cc.	2 eggs.
July 7.	72	6 cm.	1x0.5 mm.	0.1 cc.	4-5 ,,
July 11.	74	6.5 cm.	1 x 0.5 mm.	0.1 cc.	3-4 ,,

ENVIRONMENTAL VARIANTS : TEMPERATURE BAROMETRIC PRESSURE ETC.

Maximum Temp.	Minimum Temp.	Mean Temp.	Barometric Pressure.	Humidity (Saturation 100).	Rain.
103.8	78.8	91.3	29.5	28	0
111.6	80.6	96.1	29.4	25	0
113.2	95.0	104.1	29.2	29	0
112.2	84.5	98.3	29 3	56	0
111.4	87.4	99.4	29.3	25	0
110.4	88.1	99.2	29.3	45	0
101.8	79.4	90.6	29.3	65	0
103.0	84.6	93.8	29 3	56	0
97.8	81·6	89.7	29.3	67	0
98.1	88.6	93.3	29.3	67	0
102.8	83.7	93.2	29.2	63	0
91.5	77.6	84.5	29.15	87	0
90.5	79.6	85.0	29.4	72	0

SIZE AND VOLUME CHANGES OF THE OVARY.

Date of Killing.	Lizard Number	Length of the lizard from Snout to Vent.	Size of Ovary L. x B.	Volume of ovaries with oviducts.	Number of eggs in each ovary Right Left
July 16.	75	6.5 cm.	1.5 x 1 mm.	0.15 cc.	4 eggs. (slightly. big.)
July 22.	76	7 cm.	1.5 x 1.5 mm.	0.1 cc.	4 eggs.
July 26.	77	7 cm.	1.5 x 1,5 mm.	0.1 cc.	
August 6.	78	L-8.2 cm. B-2.5 cm. Tail-9 cm.	1.5 x 1.5 mm.	0.11 cc.	3-2 eggs new eggs. are formed
Aug. 7.	79	8 cm.	2 x 1.5 mm.	0.1 cc.	2-4 eggs.
Aug. 11.	80	7.5 cm.	1.5 x 1.5 mm.	0.12 cc.	3-4 eggs.
Aug. 12.	81	6.5 cm.	1 x 1 mm.	0.1 cc.	2-3 „
Aug. 16.	82	7.5 cm.	2 x 1.5 mm.	0.11 cc.	5-4 „
Aug. 20.	83	6 cm.	1 x 1 mm.	0.1 cc.	4-2 „
Aug. 26.	84	7.6 cm.	2 x 1.5 mm.	0.1 cc.	4-5 „
Aug. 30.	85	7.5 cm.	2 x 2 mm.	0.1 cc.	3 „
Sept. 5.	86	7.5 cm.	3 x 3.5 mm.	0.15 cc.	5-6 „
Sept. 10.	87	6. cm.	3 x 2 mm.	0.1 ec.	3-4 „
Sept. 11.	88	7.5 cm.	2 x 1.5 mm.	0.1 cc.	3-4 „
Sept. 19.	90	7.4 cm.	3.5 x 2 mm.	0.11 cc.	4 „
Sept. 25.	91	8 cm.	2.5 x 2,5 mm.	0.1 cc.	5-4 „
Oct. 4.	92	6.4 cm.	3.5 x 2.5 mm.	0.16 cc.	5-4 „
Oct. 9.	93	7.5 cm.	3.5 x 2.5 mm.	0.15 cc.	5 „
Oct. 14.	94	6 cm.	3 x 2.5 mm.	0.11 cc.	5-4 „
Oct. 20.	95	6.7 cm.	2.5 x 2 mm.	0.1 cc.	5 „
Oct. 28.	96	6.5 cm.	2.5 x 2 mm.	0.12 cc.	
Nov. 4.	97	7 cm.	3.5 x 2.5 mm.	0.15 cc.	4-5 ..
Nov. 11.	98	8 cm.	3.5 x 3 mm.	0.15 cc.	5 „
Nov. 20.	99	6 em.	3 x 2 mm.	0.12 cc.	6-3 „
Nov. 21.	100	7.5 cm.	3 x 2 mm.	0.15 cc.	6-7 „
Nov. 26.	101	6.5 c.m.	5 x 3 mm.	0.25 cc.	6-5 „

ENVIRONMENTAL VARIANTS: TEMPERATURE BAROMETRIC PRESSURE ETC.

Maximum Temp.	Minimum Temp.	Mean Temp.	Barometric Pressure.	Humidity (Saturation. 100).	Rain
94.0	78.8	86.4	29.3	83	0
93.8	76.6	84.8	29.2	83	0.3
84.8	77.8	81.5	29.3	95	0.1
92.2	80.4	86.3	29.4	87	0
84.4	76.6	80.5	29.2	92	0 02
82.6	77.8	80.2	29.3	91	0.52
84.5	77.6	81	29.4	79	0
91.8	80.4	86.1	29.4	74	0
95 8	78.0	86.9	29.4	71	0
94.3	80.2	87.3	29.35	72	0
95	80.7	87.9	29.45	83	0
83.5	76.6	80.1	29.5	94	0.06
86.6	75.6	81.I	29.5	83	0.26
93.7	69.4	81.5	29.5	67	0
94.4	68.9	81.7	29.7	75	0
92.4	72.9	82.7	29.6	76	0
94.1	65.7	79.9	29.7	57	0
90.7	66.5	78.6	29.6	67	0.1
84.5	58 0	71.3	29.8	63	0
84.0	53	68.5	29.7	56	0
78.7	62.6	70.7	29.6	81	0
83.0	56.7	69.9	29.8	94	0

SIZE AND VOLUME CHANGES OF THE OVARY.

Date of Killing.	Lizard Number.	Length of the lizard from Snout to Vent.	Size of Ovary L x B	Volume of ovaries with oviducts.	Number of eggs in each ovary Right Lett.
Nov. 27.	101 A	5. cm.	2 x 2 mm.	0.1 cc.	4 eggs.
Dec. 4.	102	6. cm.	2 x 1 mm.	0.1 cc.	6 ,,
Dec. 17.	103	6.5 cm.	3 x 2 mm.	0.1 cc.	4 ,,
Dec. 22.	103 A	6 cm.	2.5 x 2 mm.	0.1 cc	5 ,,
Dec. 23.	103 B	6 cm.	2.5 x 2 mm.	0.1 cc.	5-4 ,,
Jan. 12.	104	7 cm.	4 x 3.5 mm.	0.1 cc.	5-5 ,,
Jan. 13.	105	6 cm.	4 x 3 mm.	0.1 cc.	5-4 ,,
Jan. 21.	105 A	6.6 cm.	3 x 3 mm.	0.1 cc.	

ENVIRONMENTAL VARIANTS : TEMPERATURE BAROMETRIC PRESSURE ETC.

Maximum Temp.	Minimum Temp.	Mean Temp.	Barometric Pressure.	Humidity (Saturation 100).	Rain
83.4	54.5	68.9	29.8	79	0
75.1	45.8	60.5	29.8	93	0 Total rain from 1st January 38.79.

SIZE AND VOLUME CHANGES OF THE TESTES

Date of Killing.	Lizard Number.	Length of the Lizard from Snout to Vent.	Size of the Testes L. x B.	Volume of Testes with epididymis.
Jan. 2.	106 A	8 cm.	7 x 4 mm.	0.2 cc.
Jan. 13.	106 B	6 cm.	5.5 x 2.5 mm.	0.15 cc.
Feb. 21.	106	6 cm.	7 x 4 mm.	0.2 cc.
Feb. 22.	107	6 cm.	8 x 6 mm.	0.2 cc.
March 4,	108	6 cm.	7 x 4 mm.	0.2 cc.
March 5.	108 A	5 cm.	5 x 2.5 mm.	0.15 cc.
March 10.	109	6.5 cm.	7 x 2.5 mm.	0.1 cc.
March 11.	110	7.5 cm.	6 x 2.5 mm.	0.1 cc.
March 12.	111	6.75 cm.	7.5 x 4 mm.	0.12 cc.
March 16.	112	6.5 cm.	6 x 3 mm.	0 1 cc.
March 17.	113	6 5 cm.	5 x 2 mm.	0.1 cc.
March 18.	114	6.75 cm.	6 x 3 mm.	0.1 cc.
March 19.	116	5.9 cm.	6 x 2.5 mm.	0.1 cc.
March 21.	118	6.5 cm.	6 x 3 mm.	0.1 cc.
March 22.	119	6.5 cm.	6 x 3 mm.	0.1 cc.
March 23.	120	7.5 cm.	6 x 3 mm.	0.1 cc.
March 24.	121	6 cm.	6 x 2.5 mm.	0.1 cc.
April 1.	124	6.6 cm.	6 x 3 mm.	0.11 cc.
April 10	125	6 cm.	5 x 3 mm.	0.1 cc.
April 19.	126	5.5 cm.	6 x 2 mm.	0.1 cc.
April 25.	127	6 cm.	6.5 x 3.5 mm.	0.15 cc.
April 29.	129	6.5 cm.	7 x 3 mm.	0.11 cc.
April 30.	129 A	6.5 cm.	4.5 x 2 mm.	0.08 cc.
May 7.	131	6.5 cm.	4.3 x 1.5 mm.	0.08 cc.
May 8.	132	6 cm.	4 x 1.5 mm.	0.08 cc.
May 20.	135	6.5 cm.	4 x 2 mm.	0.08 cc.
May 21	137	7 cm.	4.5 x 1 mm.	0.08 cc.

ENVIRONMENTAL VARIANTS: TEMPERATURE, BAROMETRIC PRESSURE ETC.

Maximum Temp.	Minimum Temp.	Mean Temp.	Barometric Pressure.	Humidity Saturation 100.	Rain.
90.2	54.6	72.4	29.7	71	
89 8	57 6	73.7	29.7	71	0
					0
84.5	62.1	73.3	29.5	49	
82.8	59.6	71.2	29.6	30	0
86.8	56.6	71.7	29.6	32	0
92.0	60.6	76.3	29.6	51	0
					0
89.3	61.6	75.4	29.6	32	
91.8	57.1	74.4	29.7	32	0
93.3	60.9	76.9	29.6	30	0
97.8	59.7	78.7	29.6	41	0
99.1	61.8	80.4	29.7	28	0
102.0	70.1	86.0	29.4	31	0
92.9	67.4	30.1	29.7	46	0
107.4	72.8	90.1	29.4	21	0
107.8	75.2	91.5	29.4	28	0
106.8	79.4	93.1	29.5	49	0
103.8	78.8	91.3	29.5	28	0
104.3	81.6	92.9	29.45	25	0

SIZE AND VOLUME CHANGES OF THE TESTES

Date of Killing.	Lizard Number.	Length of the Lizard from Snout to Vent.	Size of the Testes L. x B.	Volume of Testes with epididymis.
June 4.	138	6 cm.	4 x 0.5 mm.	0.05 cc.
June 9.	139	6 cm.	3 x 0.6 mm.	0.05 cc.
June 16.	140	7.5 cm.	3.3 x 0.7 mm.	0.05 cc.
June 19.	141	7 cm.	4 x 0.5 mm.	0.05 cc.
July 6.	146	7.5 cm.	5 x 1 mm.	0.08 cc,
July 7.	147	6.75 cm.	3 x 0.75 mm.	0.05 cc.
July 11.	149	7.5 cm.	4 x 1 mm.	0.66 cc.
July 16.	150	6.5 cm.	2.5 x 1 mm.	0.05 cc.
Aug. 1.	151	7.6 cm.	2.5 x 1 mm.	0.05 cc,
Aug. 12.	152	6 cm.	2 x 0.75 mm.	0.05 cc.
Aug. 15.	154	7.5 cm.	1.5 x 0.7 mm.	0.05 cc.
Aug. 16.	155	6. cm.	2 x 1 mm.	0.05 cc.
Aug. 20.	156	7. cm.	3 x 1 mm.	0.08 cc.
Aug. 21.	156 A	7 cm.	3 x 1 mm.	0.08 cc.
Aug. 26.	159	7.6 cm.	4 x 1.5 mm.	0.08 cc.
Aug. 30.	150	7.5 cm	4 x 1. mm.	0.08 cc.
Sept. 2.	161	6 cm.	2.25 x 1 mm.	0.07 cc.
Sept. 3.	162	8 cm.	3 x 1.5 mm.	0.08 cc.
Sept. 4.	163	8 cm.	4.5 x 1.5 mm.	0 1 cc.
Sept. 5.	164	6.5 cm.	4 x 1. mm.	0.1 cc.
Sept. 6.	165	8 cm.	4.5 x 1 mm.	0.1 cc.
Sept. 7.	166	7 cm.	4.5 x 2 mm.	0.13 cc.
Sept. 10.	167	7.75 cm.	4 x 1 mm.	0.1 cc.
Sept. 11.	168	7.75 cm.	3.5 x 1 mm.	0.1 cc.
Sept. 17.	169	8 cm.	3.5 x 2 mm.	0.12 cc.
Sept. 23.	169 A	6 cm.	3.5 x 1.5	0.1 cc.
Sept. 25.	170	7 cm.	3.5 x 2 mm.	0.12 cc.
Oci. 7.	171	7 cm.	7 x 3.5 m.	0.2 cc.
Oct. 9.	173	9 cm.	4.5 x 2.5 mm.	0.15 cc.

ENVIRONMETAL VARIANTS: TEMPERATURE, BAROMETRIC PRESSURE ETC.

Maximum Temp.	Minimum Temp.	Mean Temp.	Barometric Pressure.	Humidity Saturation 100.	Rain.
112.2	84.5	98.3	29.3	56	0
111.4	87.4	99.4	29.3	25	0
101.8	79.4	90.6	29.3	65	0
10.30	84.6	93.8	29.3	56	0
91.5	77.6	84.5	29.15	87	0.1
90.5	79 6	85.0	29.4	72	0
94.1	78.8	86.4	29.3	83	0
84.4	76.6	80.5	29.2	92	0.02
82.6	77.8	80.2	29.3	91	0.52
84.5	77.6	81	29.4	79	0
91.8	80.4	86.1	29.4	74	0
81.5	77.6	84.5	29.5	77	0.13
94.6	78 4	86.5	29.4	77	0
95.8	78.0	86.9	29.4	71	0
94.3	80.3	87.3	29.35	72	0
95	80 7	87.9	29.45	83	0
88.1	75.6	81.9	29.4	93	0.15
91.0	75.5	83.3	29.45	94	0
86.6	75.6	81.1	29.4	83	0.26
94.0	70.3	82.1	29.7	78	0
94.4	68.9	81.7	29.7	75	0

SIZE AND VOLUME CHANGES OF THE TESTES.

Date of Killing.	Lizard Number.	Length of the Lizard from Snout to Vent.	Size of the Testes L. x B.	Volume of Testes with epididymis.
Oct. 20.	175	6.5 cm.	7 x 4.5 mm.	0.23 cc.
Oct. 27.	176	6 cm	6 x 4 mm.	0.2 cc
Nov. 1.	177	7 cm.	6 x 3 mm.	0.15 cc.
Nov. 2.	178	6.5 cm	4 x 1.5 mm.	0.1 cc.
Nov. 3.	179	6 cm.	4.5 x 3 mm.	0.15 cc.
Nov. 4.	180	6 cm.	4 x 2.5 mm.	0.15 cc.
Nov. 10.	181	6.5 cm.	5.5 x 2.25 mm.	0.2 cc.
Nov. 11.	182	6.5 cm.	7 x 3	0.25 cc.
Nov. 15.	183	8 cm	6 x 3.5 mm.	0.2 cc.
Nov. 27.	184	6 cm.	5.5 x 2.2 mm.	0.22 cc.
Nov. 29.	185	7 cm.	7 x 2 mm.	0.23 cc.
Dec. 8.	187	6 cm.	6 x 3 mm.	0.2 cc.
Dec. 13.	188	8 cm.	6 x 3 mm.	0.2 cc.
Dec. 22.	189 A.	6 cm.	7 x 3.5 mm.	0.2 cc,

ENVIRONMENTAL VARIANTS: TEMPERATURE, BAROMETRIC PRESSURE ETC.

Maximum Temp.	Minimum Temp.	Mean Temp.	Barometric Pressure.	Humidity Saturation 100.	Rain.
94.1	65.7	79.9	29.7	57	0
94 6	65.6	79.8	29.7	58	0
75.0	67.1	71.1	29.65	86	0
84.5	58.0	71.3	29.8	63	0
84.4	55.8	71.5	29.6	48	0
84 0	53.0	68.5	29.7	56	0
84.6	61.0	72.8	29.9	84	0
74.8	45.2	60	29.8	85	0

Total rain from 1st January, 39-79