The Structure of the Ovary and the formation of the Corpus Luteum in Hoplodactylus Maculatus, Gray.

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With Plates 13–19 and 4 Text-figures.

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INTRODUCTION.

The investigations into the ovarian structure of the viviparous New Zealand Gecko, Hoplodactylus maculatus Gray, recounted here, were prompted by the question of the presence or absence of a shell membrane surrounding the uterine egg. No complete account of the structure of the reptilian ovary has appeared since that of Loyez in 1906; though Thing published a paper on the zona in turtles in 1918, and Lal described cytoplasmic inclusions in the eggs of certain Indian snakes in 1933. With the advances in our knowledge of
oogenesis and in histological technique since Loyez's paper appeared, it was hoped that further light might be thrown on some of the problems of ovarian structure by another account.

The structure of the corpus luteum in Geckonidae has hitherto not been described. The accounts of this organ in various scincid lizards indicate a variability in structure uncorrelated either with generic relationships or with breeding habits, i.e. oviparity or viviparity. It was therefore of interest to discover whether an examination of the corpus luteum in a different family would assist in its classification and the determination of its evolutionary history.

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**MATERIAL AND METHODS.**

Lizards were collected at intervals around the southern and south-western coasts of the North Island of New Zealand during spring, summer, and autumn of two successive years. A number were kept in captivity during the winter, and ovaries with corpora lutea obtained from them in the following spring and summer. Owing to the irregularity of the breeding season the age of the corpus luteum is largely a matter of chance.

The following fixing fluids were used: Bouin, Zenkerformol, Zenker, Flemming, F.W.A., Maximow, and Regaud. Some of the material was double-embedded in 20 per cent. celloidin infiltrated with paraffin wax, and cut at 3 micra. The remainder was embedded in paraffin wax and cut at 5, 8, or 10 micra.

The stains used were Heidenhain's iron haematoxylin with eosin as counter-stain, Erlich's haematoxylin, Feulgen's nuclear reaction for chromatin, Wilder's silver impregnation for reticular fibres, resorcin-fuchsin, Mallory triple, Crossmann-Mallory triple, Bensley-Cowdry acid fuchsin, and methyl green.
For 'lamp-brush' chromosomes, staining with Heidenhain's iron haematoxylin for 24 hours after Flemming-without-acetic, gave good results.

**The Ovary.**

The ovary of this lizard, as Loyez (1906) states to be the case in all Geckos, has only one small localized area of germinal epithelium where oogonia are produced. The amount of stroma present is also not great, the bulk of the ovary being composed of the enlarging oocytes. Shortly after the breeding season each year, the largest oocyte in each ovary, measuring about 1 mm. in diameter, enlarges rapidly, and yolk-spheres appear in it. By the following spring its diameter is about 8 mm., and it is ready for ovulation. Thus only two eggs are ovulated annually, one from each ovary.

The germinal epithelium is composed of a layer of cells about 0.01 mm. in height, the limits of which are not clearly discernible in fixed material (fig. 2, G.E., Pl. 13). Their oval nuclei measure about 0.006 x 0.003 mm. in diameter, and contain one nucleolus. The oogonia usually sink below the surface before undergoing transformation into oocytes, and grow deeper as they become larger, until they are surrounded by follicular epithelium (fig. 2, Pl. 13). Not infrequently, however, the germinal epithelium is interrupted by the presence of a small oocyte in it; but the oocytes must migrate inwards before they become surrounded by follicular epithelium. Occasionally several enlarging oocytes lie together; but usually each is separated from its neighbours by cells of the germinal epithelium which have migrated below the surface, or by stroma cells. This part of the ovary, where oogonia are developing and undergoing transformation into oocytes, will be referred to as the germinal bed.

The cell membrane of an oogonium which is enlarging to form an oocyte becomes very distinct. The cytoplasm stains very lightly, and a crowd of mitochondria in the form of short, straight, or bent rods of varying thickness form a crescent-shaped mass beside the nucleus in oocytes of about 0.02 mm. in diameter. Also beside the nucleus, among the mitochondria, is a centrosphere surrounded by clear cytoplasm, as Loyez and
others have described. The nucleus is spherical and usually eccentric.

When the nucleus of the enlarging oogonium reaches 0.008 mm. in diameter (cell about 0.01 mm.) the chromosomes have assumed the form of blob-like bodies at the periphery (fig. 1, Pl. 13). The nucleolus is usually more or less central. This condition gives place to the leptotene stage. Zygotene follows normally, and, after the pachytene stage, in which the threads do not appear to be as definitely attached at one side as in typical cases, the diplotene stage is entered upon. By this time the oocyte has reached a diameter of 0.024 mm., and the nucleus 0.014 mm. The chromosomes have become granulated, as typically occurs, and they stain more lightly than at pachytene (fig. 2, X., Pl. 13). The filaments then begin to assume the form of ‘lamp-brush’ chromosomes (fig. 2, Pl. 13), which cease to react to Feulgen’s stain. At the same time the nucleolus enlarges and becomes vacuolated (fig. 2, NL., Pl. 13), and several small nucleoli appear in addition.

Loyez has described and figured lamp-brush chromosomes in several lizards, but failed to recognize this as the continuation of the diplotene stage. She considered that a ‘reticulum’ characteristic of the resting nucleus was formed after early diplotene, and gave rise to the lamp-brush chromosomes. The position of the chromosomes is unrelated to that of the nucleoli, and Loyez’s observation that the position of the nucleoli had a definite relation to that of the ‘reticulum’ was probably due to an artifact.

As growth continues a rearrangement of the lamp-brush chromosomes occurs, so that they are more or less evenly distributed in the nucleus when the oocyte reaches about 0.07 mm. in diameter, and the nucleus 0.03 mm.

At the beginning of the diplotene stage the cytoplasm of the oocyte increases rapidly in quantity and density. As in a number of other vertebrates, the centrosphere disappears before the diplotene stage. The mitochondria scatter throughout the increasing cytoplasm, although for a time a crescentic region where they are most numerous is distinguishable by the nucleus.
OVARY OF HOPLODACTYLUS

In an oocyte of 0-08–0-1 mm., with a nucleus measuring 0-035 mm. in diameter, all the space between the lamp-brush chromosomes is occupied by a very finely granular acidophil nucleoplasm (fig. 2, Y., Pl. 13). The cytoplasm of oocytes of this size is becoming alveolar in character, and appears to contain a loose network of fine twisted and branching threads, which becomes more and more marked with growth (figs. 2 and 3, Pl. 13).

Small vacuoles begin to appear near the periphery of the oocyte of 0-15–0-2 mm. in diameter (fig. 8, Pl. 14). They are occupied by small fat globules which Loyez states to be derived from the cells of the follicular epithelium, which contain fat (fig. 6, Pl. 14). It travels into the oocyte through the tubular prolongations of the large epithelial cells. The periphery of the oocyte contains, also, small, irregular, dense patches, which Loyez suggests to be due to substances, other than fat, passed in from the follicular epithelium. In addition, the mitochondria have largely become aggregated at the periphery, although many remain scattered throughout the cytoplasm.

 Except for an increase in the number of small vacuoles containing fat, no changes occur in the cytoplasm and its inclusions until after the oocyte has reached 1 mm. in diameter; but a number of changes occur in the nucleus during this time. When the germinal vesicle grows beyond 0-035 mm. in diameter, the chromosomes are no longer distributed throughout it, but form a spherical group in the centre 0-035 mm. across. This is due to the chromosomes maintaining their earlier arrangement unaltered by the growth of the nucleus (fig. 3, Pl. 13). This feature is apparently peculiar to reptiles, since it has not been recorded in cyclostomes, amphibia, or birds. In each of these classes the lamp-brush chromosomes are spread throughout the nucleus.

In oocytes between 0-1 and 1-0 mm. in diameter there are several generations of nucleoli. Oocytes of 0-1–0-3 mm., with nuclei of 0-06–0-1 mm., contain up to five or six large vacuolated nucleoli situated among the chromosomes. The growth of the vacuoles, of which there are several in each nucleolus giving it a morula-like shape, causes an increase in the size of the nucleolus,
which may reach 0.014 mm. in diameter. As a result the walls become extremely thin and almost achromatic. The nucleoli then disappear by the bursting of the walls. This mode of disappearance of nucleoli has been described in oocytes of Triton by Lubosch (1902). The numbers of large, multi-vacuolated nucleoli are maintained and increased by the growth and vacuolation of small nucleoli which constantly appear in the nucleus.

Some of the nucleoli at this period resemble those called by Loyez (1906) 'nucléoles triples' and 'nucléoles avec corps accessoires'. This appearance is due to the chance juxtaposition of one or more small nucleoli against a larger one, or to the viewing of one nucleolus partly over another. The eccentric position of vacuoles accounts for the appearance of forms like those Loyez figures with a dense crescentic thickening at one side. No evidence of multiplication by budding as she describes has been observed. All the nucleoli seem to appear first as minute bodies which grow and become vacuolated.

The large multi-vacuolated nucleoli have all disappeared from oocytes of 0.35 mm. in diameter. In oocytes of 0.3–0.6 mm. with nuclei of 0.1–0.2 mm., several nucleoli of about 0.004 mm. in diameter are present (fig. 8, NL, Pl. 13). These nucleoli stain well, in contrast with the multi-vacuolated ones, contain only a single vacuole, and do not grow beyond 0.004 mm. or become achromatic. They usually lie near the border of the group of chromosomes. The number of small or minute nucleoli present in the nucleus is greater during this period than the last.

The larger vacuolated nucleoli all disappear from oocytes over 0.6 mm.; but there is no evidence of their being passed into the cytoplasm as Loyez and others have described in the lower vertebrates. In oocytes of 0.6 mm. and over the small nucleoli increase enormously in number. They are no longer confined to the central part of the nucleus among the chromosomes, although for a time they are absent at the periphery.

The lamp-brush chromosomes in oocytes of about 0.6 mm. in diameter become more closely arranged, so that the group formed by them in the centre of the nucleus is only about 0.030 mm. across. In oocytes of 0.8 mm. the lamp-brush
chromosomes have become more compact, and condensation continues until, in oocytes of about 0.95 mm. in diameter with nuclei of 0.31 mm., the chromosomes have returned to the form of beaded threads (fig. 4, Pl. 13). They are still in the diplotene stage.

The small nucleoli are now scattered thickly throughout the nucleus. Those near the periphery tend to be larger than those near the centre (fig. 4, Pl. 13).

In an oocyte of 1.1 mm. in diameter the bivalent chromo-

osomes are rod-like. They become more closely packed, and in an oocyte of 1.35 mm. in diameter with a nucleus of 0.82 mm. the group of chromosomes is only 0.015 mm. across (Text-fig. 1). Small nucleoli remain thickly scattered throughout the nucleus.

In the 1-mm. oocyte the cytoplasm becomes much denser near the centre. Frequently irregular arms of the denser cytoplasm extend towards the periphery from the central part. A yolk-nucleus was observed in the dense cytoplasm of an oocyte of 1.08 mm. in diameter (fig. 9, Pl. 14). It is a small vacuolated body, 0.025 × 0.015 mm. in diameter, stains with haematoxylin, and resembles those of this type described by Loyez. No trace of a centrosphere or yolk-nucleus is distinguishable between the early prophase stage and the present one.
Shortly after this, in oocytes of about 1·1 mm. in diameter, a zone of vacuoles occurs at a distance of about one-third of the radius from the periphery, outside the central dense cytoplasm. At the same time extremely minute, basophil yolk-spheres appear at the periphery, and extend in among the vacuoles. The spheres originate in minute vacuoles which they usually fill completely. The vacuoles forming the zone mentioned above do not contain yolk-spheres. To them is probably due the arrangement of the yolk in the ripe egg, between fairly large vacuoles. There is a gradual transition between the minute yolk-spheres at the periphery and those among the vacuoles, which are larger and fewer in number. At the centre of the oocyte the dense cytoplasm is becoming markedly alveolar. The angular yolk elements observed by Loyez do not occur here, and at this stage acidophil yolk elements are indistinguishable.

The vacuolated zone extends inwards, and the vacuoles become larger. In an oocyte of 1·5 mm. in diameter the zone extends through the middle third of the egg. The yolk-spheres are now much more numerous; but away from the periphery they are still scarcer and larger. The peripheral spheres vary in size from those just visible to those of 0·5 micra in diameter. Independently of size some are acidophil, and others, which occur more frequently, are basophil.

In the denser cytoplasm inside the zone of vacuoles, extremely minute yolk-spheres, like those at the periphery in the 1·1-mm. oocyte, are now appearing in fair number. There is no transition in size between these and the larger spheres in the vacuolated zone.

In an oocyte of about 6 mm. in diameter, vitellogenesis is nearing completion. The large oocyte composes the greater part of the ovary, which shows a considerable increase in size since the close of the last gestation period. The germinal bed and smaller oocytes form low protrusions on the cephalic side of the 6-mm. oocyte. The germinal disk is situated at the opposite side, unlike the frog. It is nearly 2 mm. in diameter and its greatest depth is 0·8 mm. At its margin the germinal disk is continuous with a narrow layer of cytoplasm, containing fine yolk-spheres,
which lies immediately beneath the zona. Similar fine yolk-spheres are thickly scattered, without definite arrangement, in the germinal disk. The nucleus, which is eccentric throughout the development of the oocyte, but remains some distance from the periphery even after vitellogenesis has begun, is now situated just above the deep border of the germinal disk. The latebral neck passes down from the germinal disk below the nucleus.

The nucleus is slightly kidney-shaped, but shows no increase in size over that of the 1·35-mm. oocyte at the beginning of vitellogenesis. It measures about 0·37×0·26 mm. The rate of increase in the size of the nucleus is greater than that of the oocyte until the onset of the diplotene stage. From then until the oocyte reaches 1·3 mm., at the beginning of vitellogenesis, the increase of the nucleus is approximately proportional to that of the oocyte. Thereafter the oocyte increases enormously, while the nucleus remains stationary. The only change in the nucleus is the crowding of the bivalent chromosomes to form a small spheroidal group, measuring 0·006×0·012 mm., near the centre of the nucleus. The very numerous small nucleoli remain as before in the nucleoplasm.

The yolk, apart from that in the germinal disc and peripheral zone, is arranged around and between numerous vacuoles of about 0·03 mm. in diameter (fig. 13, Pl. 15). This arrangement is interrupted at intervals in most eggs, causing the yolk to be divided into two or three zones (Text-fig. 2). Owing to the shrinkage of fixed material, these zones appear to be separated by a thin layer where the yolk is sparsely scattered. The zones are no doubt homologous with the alternating layers of fine and coarse yolk described by Sarasin (1889) for Lacerta agilis, and attributed by him to periods of greater activity in yolk-formation, governed by changes in nutrition, or other factors affecting the parent. The yolk-spheres vary from 0·005–0·01 mm. in diameter.

The latebra consists of clear alveolar cytoplasm, containing near the border many large vacuoles similar in size and arrangement to those among the yolk (fig. 13, Pl. 15). It is pear-shaped, directed obliquely to the plane of the germinal disk, and lies
somewhat excentrically near the germinal disk. The narrower end, which is nearer to the germinal disk, is continuous with the latebral neck, which follows a somewhat winding course, as Sarasin observed in Lacerta, to the germinal disk. The latebral neck consists of cytoplasm which is markedly alveolar, and contains fine yolk-spheres that merge with the coarser yolk around them. Bordering the vacuoles near the margin of the latebra, and also between them, are minute yolk-spheres. There is a gradual transition of these minute spheres into the coarser ones farther out, where the cytoplasm is not discernible.
The Follicular Epithelium.

The first indication of the formation of follicular epithelium around the oocyte occurs when its diameter is about 0.024 mm., corresponding with the onset of the diplotene stage in the nucleus. Cells of the germinal epithelium which have migrated inwards, and lie scattered among the enlarging oocytes, begin to surround the latter. Occasionally a degenerating nucleus has been observed in an oocyte of this size, containing also an apparently healthy nucleus. Whether this is due to the fragmentation of a degenerating nucleus, or to the combination of two oocytes and the subsequent degeneration of one nucleus, as Balfour (1878) affirms happens constantly in the dogfish, is obscure.

The follicular epithelium is almost complete when the diameter of the oocyte reaches 0.045 mm. The cells are roughly cubical and measure about 0.008 mm. in thickness. By the time the oocyte is 0.07 mm. in diameter the follicular epithelium is becoming double. The oocyte has moved to one side of the germinal bed, always the same side, and is in contact with the bulk of the stroma on its inner side (fig. 2, Pl. 13). This position obviates the destruction of the stroma and germinal bed during the subsequent enlargement of the oocyte.

Certain epithelial cells here and there now become enlarged. The follicular epithelium at this stage is very irregular in character, consisting in some places of a double layer of small cells, in others of a single layer, and interrupted now and again by an enlarged cell (fig. 2, Pl. 13).

Loyez considers the changes undergone by the enlarging follicular epithelial cells to be similar to those in the development of the oocyte from the oogonium. In this lizard, however, there are important differences, at least in regard to the nucleus. The nuclei of the small follicle cells resemble those of the oogonia in the germinal epithelium. As they enlarge the chromatin ceases to react to Feulgen's stain; but no stage comparable with leptotene or zygotene occurs. The pre-meiotic phase of blob-like, contracted chromosomes at the periphery of the oogonium about to give rise to an oocyte is also absent from the enlarging
follicular cells. During the growth of the nucleus the nucleo-
lus increases in size and becomes vacuolated, resembling that 
of the oocyte shown in fig. 2, Pl. 13. The nucleus grows to a 
diameter of about 0.02 mm.

A crowd of rod-shaped mitochondria appear in the cytoplasm 
of the enlarging epithelial cell, as in the young oocyte. The 
cytoplasm stains better than it does in the transforming 
ogonium. The centrosphere, observed by Thing (1918) in the 
epithelial cells of turtles, is not distinguishable here. An oocyte 
of about 0.13 mm. in diameter is completely surrounded by 
a layer of enlarged cells, each about 0.03 mm. in diameter. A 
layer of small cells remains immediately outside the egg mem-
brane, the cells occupying the spaces between the protruding 
ends of the large cells. Another layer of small cells lies outside 
the larger ones, just beneath the theca. Loyez records the 
ocurrence of mitoses only in the inner layer of small cells; but in 
this lizard they occur in the small cells of both the inner and 
outer layers. Division is more frequent in the inner layer, as 
it is from this layer that all the larger cells are derived.

The follicular epithelium of an oocyte of 0.3 mm. in diameter 
exhibits the typical character and arrangement found through-
out the functional period of the epithelium. The large cells 
have become flask-shaped, as Loyez has described, having their 
long axes directed radially and their broad ends outward 
(fig. 12, L.F., Pl. 15). They measure about 0.20×0.03 mm. but 
may be narrower and longer. The narrow end is prolonged 
to form a small tube which pierces the zona in a funnel-shaped 
opening, resembling those figured by Loyez (fig. 5, M.T., 
Pl. 14). The knob-like enlargement of these tubes at the yolk-
surface, which Thing observed in turtles, is absent. The nucleus 
lies near the outer end of the cell. It is unchanged, except for 
an increase in the size of the nucleolus (fig. 12, N.t., Pl. 15). The 
walls of the nucleolus become very thin, like those in oocytes of 
0.06–0.08 mm., and may burst, sometimes discharging a few 
small nucleolus-like granules contained in the vacuoles, as Loyez 
observed. There may be two or three vacuolated nucleoli, 
resulting from the growth and change of small ones. The cycle 
of changes which these nucleoli undergo in the oocyte does not
appear to occur usually in the epithelial cells. The cytoplasm near the nucleus contains a crowd of mitochondria, and also small fat-globules (fig. 6, F.G., Pl. 14). Sometimes large cells in the follicular epithelium of oocytes over 1 mm. in diameter become packed with fat-globules, the nucleus degenerates, and the tubular connexion with the oocyte is lost.

Small undifferentiated cells still lie between the swollen ends of the large epithelial cells, which occupy the meshes of a network of small cells when viewed in tangential section (fig. 12, S.F., Pl. 15). Between the narrow inner ends of the large cells is an irregular layer of intermediate cells derived from the inner layer of small cells in the same way as the large cells (fig. 9, I.F., Pl. 14). Their nuclei, which do not react with Feulgen's stain, contain a vacuolated nucleolus, while the cytoplasm contains mitochondria and some minute fat-globules. They are an intermediate stage in the formation of the large cells, and as the surface of the oocyte increases they grow to become part of the layer of large cells. Against the zona there remains an incomplete layer of small cells, interrupted by the presence of intermediate cells. The follicular epithelium of this lizard is thus of the same type as that of the Geckos examined by Loyez. Cytoplasmic connexions between the follicle cells, as figured by Eimer (1872) and also observed in turtles by Thing, occur here. They may branch, and in the case of the large follicle cells they may be comparatively coarse.

The follicular epithelium of an oocyte near the close of vitellogenesis, measuring 6-8 mm. in diameter, consists, as a result of shrinkage of the larger cells, of a single layer of small cells only (fig. 7, F.C., Pl. 14). The cells have a shrivelled margin, and the quantity of cytoplasm is small, with the consequent occurrence of intercellular spaces. The oval nuclei of the cells measure about 0.008 x 0.004 mm., and contain one or two small nucleoli.

The Theca.

The theca is derived from the stroma when the oocyte, surrounded by follicular epithelium, leaves the germinal bed, as Loyez (1906) described. It consists of fibroblasts concentrically
arranged (fig. 12, T., Pl. 15). Elastic fibres are numerous, and reticular fibres are present, in addition to collagen fibres. It is a simple layer, not differentiated into theca interna and externa until late in the development of the oocyte.

The theca of the ripe or nearly ripe oocyte, 6–8 mm. in diameter, measures only about 0.05 mm. in thickness. Surrounding the follicular epithelium is a membrana propria, distinct from the rest of the theca. It is composed of flattened fibroblasts (fig. 7, M.P., Pl. 14). Immediately outside this in some places, especially between capillaries, are cells arranged as a single layer, having oval nuclei and no fibres among them (fig. 7, T.I., Pl. 14). Although this layer is so discontinuous, the gaps being greater than the areas where it is present, it seems nevertheless to constitute a rudimentary theca interna comparable with that of mammalia. The theca externa consists of flattened fibroblasts, and resembles the undifferentiated theca. Small capillaries run beneath the basement membrane, and large flattened vessels lie outside them (fig. 7, B.V., Pl. 14).

The Vitelline Membranes.

As in other vertebrates, the egg is surrounded by an egg membrane, which is derived from the primitive membrane of the transforming oogonium. Between this and the zona pellucida there is a striated layer (fig. 7, Pl. 14). The egg membrane is called by Loyez the internal membrane, while the zona pellucida is called vitelline membrane. Both Loyez (1906) and Thing (1918) designate the striated layer as the zona radiata. Thing includes the striated layer with the dense outer layer under the name zona pellucida.

In transverse section the striated layer appears to consist of thin, radially-directed, rod-like protuberances of the zona pellucida (fig. 7, Z.R., Pl. 14). Small spaces of varying width are present between the rods, which thicken at their junction with the zona pellucida. In tangential section the striated layer resembles a layer of small vacuoles.

The zona pellucida is composed of a dense substance, apparently homogeneous, even in tangential section. It is pierced by prolongations of the large follicular epithelial cells, but does
not appear striated in the intervals, in fixed material. Loyez observed radial striations in the zona pellucida of one or two Geckos, however, and Thing found such striations in living eggs of turtles. The striations, as Loyez suggests, are probably produced by the prolongations of follicular epithelial cells.

The zona pellucida first appears as a few irregular thickenings between the egg membrane and the follicular epithelium of an oocyte of about 0.07 mm. in diameter, around which the single-layered follicular epithelium is complete (fig. 3, Pl. 13). The thickenings follow the contour of the follicle cells, and often jut out to a point between the ends of adjacent cells, as Thing observed in turtles. There are, however, no intercellular channels filled with intercellular substance as in turtles, and the epithelial cells lie against the egg membrane before the formation of the zona. The inner borders of the thickenings are smooth and follow the contour of the egg membrane. They have the appearance of a homogeneous secretion.

The thickenings increase and join together as the follicle cells multiply and enlarge. In this way the zona becomes complete round an oocyte of about 0.15 mm. in diameter; but for some time after this it is interrupted by the presence of small follicle cells of the inner layer engulfed in it at frequent intervals. These cells lie against the egg membrane. Some of them occasionally sink into the oocyte, are isolated from the epithelium by the growth of the zona, and subsequently degenerate. Loyez observed small degenerating follicle cells in the cytoplasm of the oocytes of other lizards.

Thing describes the structure of the zona as a primary network with a superimposed secondary network; but this cannot be traced here. The apparently homogeneous cuticular substance of the zona is, however, interrupted by prolongations of the large follicle cells, and in addition there are a number of minute pores in it, which may contain cytoplasmic connexions from the smaller follicle cells. These do not show in the fixed material, although the prolongations of the large cells are distinct in tangential sections.

The striated layer is evident in an oocyte of about 0.15 mm.
as a very thin, clear layer between the zona pellucida and the egg membrane, in sections stained with Crossmann-Mallory triple stain, which stains the egg membrane purple and the zona pellucida green.

The zona of an oocyte of 0.3 mm. in diameter varies considerably in thickness, since the outer surface still follows the irregularities of the follicular epithelium; but it averages 0.002 mm. Its inner face in transverse section presents a scalloped outline, due to its being perforated at intervals by the tubular prolongations of the enlarged epithelial cells, as Loyez and Thing observed. It becomes very thin around these perforations.

As the zona pellucida thickens it becomes denser, and the egg membrane also becomes stouter and stains more deeply. The zona pellucida of an oocyte of 0.7 mm. in diameter measures 0.003 mm. and the striated layer 0.001 mm. in thickness. The radial striations are now distinguishable in the latter. They stain green like the zona pellucida with Crossmann-Mallory triple stain, and are thus distinguished from cytoplasmic material which stains pink or purple. The zona has lost its scalloped outline, the perforations for the epithelial cell connexions with the oocyte being funnel-shaped (fig. 5, M.T., PL 14). As Loyez pointed out, the narrower ends of the openings are next to the epithelium. The egg membrane lines the funnel-shaped openings.

The zona pellucida increases to 0.004 mm. in thickness round an oocyte of about 1 mm. in diameter; but the striated layer shows no increase (fig. 12, PL 15). Owing to refraction of light there appears to be a dark layer in the zona next to the striated layer in fig. 9, PL 14. This is not a true distinction.

When the oocyte reaches 6 mm. in diameter the zona pellucida is reduced in thickness and measures just over 0.001 mm., while the striated layer has greatly increased and measures 0.005 mm. (fig. 7, PL 14). The egg membrane remains on its inner surface, but is rather indefinite and has a granular appearance. It is more distinct in the uterine ovum. The perforations for prolongations of the large epithelial cells are apparently lost. The reduction in thickness of the zona
pellucida as the egg ripens is in contrast with the behaviour in turtles, monotremes, and other vertebrates.

The formation and appearance of the zona pellucida in this lizard confirms the statement made by Thing, as a result of her researches in turtles, that it is a cuticular structure. The striations of the striated layer are here clearly shown by Crossmann-Mallory triple staining to be composed of the same cuticular substance. They are, therefore, not cytoplasmic in origin and cannot be prolongations of epithelial cells.

Follicular Atresia.

Two atretic follicles are present in only a single ovary among fifteen examined. The cause of atresia in this case appears to be that two oocytes have enlarged together, and considerable vitellogenesis has occurred in both. Normally only one oocyte attains maturity at any one time in the ovary. Loyez remarks upon the rarity of atretic follicles in the Gecko, Platydactylus muralis, in which she found none, though Mingazzini (1893) observed atresia in Platydactylus mauritanicus.

One atretic follicle is 3 mm. in diameter, and the other, 4–5 mm., is irregular in shape. Atresia is more advanced in the latter. In neither is there any trace of the germinal disk or the latebra.

The theca of the 3 mm. follicle is thinner than that of a healthy oocyte of this size and contains few capillaries. The zona has completely disappeared. The follicular epithelium has given rise to a festooned layer of giant cells with bulging free ends protruding into the yolk. Their cytoplasm is extremely alveolar, and the nuclei are irregular in shape and contain vacuolated nucleoli. The cells contain masses of yolk-spheres, particularly round the margins, in various stages of dissolution. The normally basophil yolk-spheres become acidophil and vacuolated. In a few places fibroblasts from the theca have grown in between the giant cells. No cells have yet migrated into the yolk, but the spheres have amalgamated to form irregular vacuolated masses between the large vacuoles present in normal yolk.
The theca of the larger follicle has become very thin, due, apparently, to the inward migration of fibroblasts to invest the giant cells, and penetrate the yolk. Capillaries are small and very few in number. The giant cells have become reduced to half their former size and form a layer two or three cells deep at the periphery of the egg. Their cytoplasm is denser than before, owing partly to absorption of the broken-down yolk. Cell boundaries are often difficult to distinguish.

Both epithelial cells and fibroblasts from the theca have penetrated into the deep yolk. Many of the large vacuoles are now occupied by epithelial cells with extremely alveolar cytoplasm. Numbers of polymorphonuclear and mononuclear leucocytes are present in the masses of yolk near blood-vessels, which are beginning to penetrate into the yolk.

The course followed in regression in this species seems to be first the disappearance of a distinctive theca, then the absorption of the yolk by giant cells and later by leucocytes also. Finally, the giant cells become reduced and disappear, the position of the atretic follicle being marked for a time by fibroblasts which grow in from the theca.

**DISCUSSION.**

The occurrence of a prolonged diplotene stage with the formation of 'lamp-brush' chromosomes during the growth period of the oocyte has previously been observed in all classes of vertebrates having large yolky eggs, with the exception of reptilia. The description given above of this phenomenon in a reptile therefore completes the list. That precisely similar nuclear changes occur in Scincidae and Anguidae is shown by the figures given by Loyez (1906).

Among cyclostomes, Okkelberg (1921) described the ovary of the brook-lamprey, and states that the diplotene stage persists throughout the early part of the growth period and probably up to the time of maturation. He finds that the nucleus becomes very large and the chromatin material may be scattered throughout its whole extent, and therefore it is difficult to follow the history of the chromosomes during the growth period. There is little doubt from this description, and a comparison
of his figures 59, 60, and 62, that the chromosomes have assumed the lamp-brush form.

In Elasmobranchs Buckert (1892) described and figured typical lamp-brush chromosomes during the growth period of the oocyte.

King (1908) described and figured lamp-brush chromosomes in Bufo among Anura during the growth period of the oocyte, but did not recognize this as the diplotene stage. Carnoy and Lebrun (1897) had previously observed lamp-brush chromosomes in oocytes of Rana, but mistook their origin.

In Triton among Urodeles Koltzoff (1938) has given a detailed and well-illustrated account of the lamp-brush chromosomes in the nucleus of the enlarging oocyte.

Crew (1933) noted the persistence of the diplotene stage and the occurrence of lamp-brush chromosomes in the growing oocyte of the domestic fowl. Koltzoff has described this phase in the chick and the pigeon.

Flynn and Hill (1939), in their paper on the growth of the ovarian ovum in the Monotreme, have designated as pseudo-chromosomes what are evidently lamp-brush chromosomes in the diplotene stage.

The observations of Loyez and the present account show that the Lacertilia are distinguished from the other classes in that the lamp-brush chromosomes have condensed to bivalent rod-like chromosomes before the yolk is deposited, and for some time before this they are confined to the more central part of the nucleus.

THE UTERINE OVUM.

The ripe ovarian egg measures about 8 mm. in diameter; but in utero the ovum is oval in shape, due, no doubt, to the pressure of the muscular uterine wall which fits closely about the soft egg membranes. It measures 9 × 7 mm.

Bounding the yolk is a very thin membrane which seems to be the egg membrane (fig. 10, V.M., Pl. 15). It is much more distinct than in the 6-mm. oocyte. Immediately outside it is a thin and very delicate striated layer which measures only 0.001–0.002 mm. in thickness (fig. 10, Z.R., Pl. 15). This apparently represents the striated layer of the 6-mm. oocyte.
Attached to it is a dense membrane varying in thickness from about 0.003–0.01 mm. (fig. 11, Pl. 15). This is bilaminar. The inner layer is slightly denser, thinner, and constant in thickness, measuring 0.002 mm. It is apparently the zona pellucida which has doubled its thickness in the 6-mm. oocyte. This interpretation is supported by the fact that its inner face shows minute denticulations, like the thickened terminations of the striations of the striated layer in the large ovarian oocyte.

The outer part of the bilaminar membrane is formed by the shell membrane which varies in density over different parts of the egg. In the thicker places the density is less. It is eosinophil, having the appearance of fine felted fibres embedded in a matrix, as Giersberg (1922) observed to be the appearance of the shell membrane in other lizards. He has shown that no albumin is secreted round the ova of lizards. Occasional small cells are present between the shell membrane and the zona. They resemble follicular epithelial cells, and probably remained attached to the zona at ovulation. Small globules of a secretion similar in appearance to the shell membrane are tangled here and there in the cilia of the uterine epithelial cells.

Among the peripheral yolk-spheres, which are now only slightly smaller than the rest, is a little cytoplasm and some coagulum. Vitellogenesis has ceased first, as it began, at the periphery, for no minute yolk-spheres, like those still occurring in the latebra, are present here. The yolk, except at the periphery, is arranged between large vacuoles, as in the 6-mm. oocyte (fig. 13, Pl. 15); but no zones of growth are distinguishable. They may have been destroyed by the alteration in shape of the uterine ovum, and by the distortion due to sectioning; but Sarasin (1883) states that they vary in distinctness in different eggs.

The latebra, as in the 6-mm. oocyte, is pear-shaped, but its long axis is now parallel to the plane of the germinal disk, so that the neck traverses a curve of about 90° as it winds up to the disk (fig. 13, L.N., Pl. 15). The latebra lies anteriorly to the germinal disk (in the parent) and towards the remote side of the egg. The displacement may be due to the change of shape undergone by the uterine egg; but Sarasin often found the
latebra excentric in *Lacerta agilis*. The character of the latebra differs somewhat from that of the 6-mm. ovum. It has a marked alveolar appearance, but is much denser than before, and resembles the cytoplasm of oocytes just before the onset of vitellogenesis (fig. 13, Pl. 15). There is a quantity of coagulum scattered in the yolk, mainly at the periphery and near the latebra, and this seems to be partly responsible for the increase in density of the latebra. The central part of the cytoplasm is no longer vacuolated, while the vacuoles near the border are smaller than those of the 6-mm. oocyte, and do not exceed 0·005 mm. in diameter. The transition between the latebra and the yolk is therefore sharper than before, as is expected after the cessation of vitellogenesis. Fine yolk-spheres are present between the marginal vacuoles of the latebra; but this layer is thin. The innermost spheres are acidophil and very fine, while the rest are basophil. The latebra differs from that of the oviducal egg of *Lacerta agilis* in that fine yolk-spheres are present throughout the latter.

The core of cytoplasm in the latebral neck is dense where it leaves the latebra, is about 0·8 mm. in diameter and is surrounded by fine yolk extending out for at least 0·5 mm. (fig. 13, L.N., Pl. 15). Both the density and the diameter of the cytoplasmic core diminish as it winds upwards. About half-way up it is only 0·02 mm. across, and is very vacuolated and stains lightly. The diameter increases again as it approaches the germinal disk.

The germinal disk lies dorsally in the uterus and is oval, as Nicolas (1900) noted in *Anguis fragilis*. It measures approximately 2·3 x 2·7 mm. in diameter, while its greatest depth is 0·8 mm. It extends downwards, plug-like, at its centre, as in *Ornithorhyncus*, and into this 'axial plug' passes the latebral neck. The core of clear cytoplasm in the latebral neck can be traced to the centre of the axial plug, where it disappears. The cytoplasm of the germinal disk is closely and uniformly packed with very small yolk-spheres which pass gradually into the coarser yolk at the lower side of the disk. It thus differs from that of *Anguis fragilis* (Nicolas, 1900) in containing no cytoplasm more or less clear of yolk-
spheres, except sometimes a thin fringe beneath the egg membranes.

Oppel (1892) observed at the periphery of eggs of Anguis fragilis patches of cytoplasm containing fine yolk-spheres, resembling the germinal disk, but separated from it. Nicolas found such patches in a few of his eggs. In the egg under description such a patch is present well round to the side of the egg. It is small, being only 0.2 mm. across. It seems to be of merely accidental occurrence, due to some irregularity of vitellogenesis at that place, and of no significance.

Probably owing to abrasions of the surface in sectioning no polar bodies were found.

**Nuclei in the Germinal Disk.**

Approximately at the centre of the disk, and 0.06 mm. beneath the surface, are the male and female pronuclei (fig. 11, Pl.). They lie in contact, one deeper than the other, the deeper one slightly indented by the other and with their long axes parallel to the surface. They are, therefore, in position for the appearance of the spindle of the first division. Their arrangement is like that described by Nicolas at a certain stage in Anguis fragilis. The deeper one measures 0.015 × 0.008 mm. in diameter, and the other 0.013 × 0.01 mm. Both are in the resting condition, being vesicular and having one conspicuous deeply staining nucleolus. The nuclear membranes stain deeply and are well defined. It is impossible to distinguish the male pronucleus from the female.

At the surface of the germinal disk, opposite the pronuclei, the entrance cone of the spermatozoon is plainly evident as a portion of dense cytoplasm containing no yolk-spheres. A small area of cytoplasm round the pronuclei is also free from yolk-spheres (fig. 11, Pl.). There is, however, no trace of a centrosome or aster. The tail of the spermatozoon cannot be identified; but its path is visible owing to the presence of some vacuoles, and the partial absence of yolk-spheres from it. Nicolas found that the track of the spermatozoon was often vacuolated. It has the form of a parabolic arc.

Six other nuclei are present in the germinal disk, three
OVARY OF HOPLODACTYLS

situated anteriorly and three posteriorly to the pronuclei (Text-fig. 3). They are supernumerary sperm nuclei, the entrance cones of which are usually visible nearby. Sometimes the track of the spermatozoon is also distinguishable, in which case it is a parabolic arc. The following table gives the position and measurements of these nuclei, lettered as in Text-fig. 3:

<table>
<thead>
<tr>
<th>Nucleus</th>
<th>Measurements in mm.</th>
<th>Distance below Surface</th>
<th>Distance Anterior (+) or Posterior (—) to Pronuclei</th>
<th>Distance Right (+) or Left (—) of Mid-line</th>
<th>Position of Entrance Cone</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0·12×0·01</td>
<td>0·03</td>
<td>+0·95</td>
<td>—0·7</td>
<td>doubtful</td>
</tr>
<tr>
<td>B</td>
<td>0·12×0·009</td>
<td>0·034</td>
<td>+0·4</td>
<td>+0·9</td>
<td>opposite</td>
</tr>
<tr>
<td>C</td>
<td>0·01×0·01</td>
<td>0·055</td>
<td>+0·34</td>
<td>—0·1</td>
<td>opposite</td>
</tr>
<tr>
<td>D</td>
<td>0·012×0·009</td>
<td>0·03</td>
<td>—0·2</td>
<td>+0·2</td>
<td>0·22 mm. to left</td>
</tr>
<tr>
<td>E</td>
<td>0·009×0·009</td>
<td>0·05</td>
<td>—0·35</td>
<td>—0·45</td>
<td>0·5 mm. to right</td>
</tr>
<tr>
<td>F</td>
<td>0·01×0·008</td>
<td>0·036</td>
<td>—1·24</td>
<td>—0·75</td>
<td>0·1 mm. anterior</td>
</tr>
</tbody>
</table>

Diagram showing situation of the supernumerary sperm nuclei in the germinal disk. P.N., male and female pronuclei; A—F, supernumerary sperm nuclei. ×12.

These nuclei all closely resemble each other. They are in the resting state, being vesicular and containing one deeply-staining nucleolus (fig. 8, Pl. ). Like the pronuclei, each is situated in a small area of dense cytoplasm free from yolk-spheres. Their long axes are parallel to the surface. In two cases a short length of the tail is distinguishable. In one, a very dense portion of cytoplasm, possibly representing the middle piece of the spermatozoon, lies against the nucleus,
and from the opposite side of this cytoplasm the tail extends between yolk-spheres for about 0.02 mm. (Text-fig. 4, T.). In the other the tail lies partly in the clear area of cytoplasm round the nucleus, and curves out between the yolk-spheres. It can be traced for about 0.04 mm. The tails are very fine and, as Nicolas found, stain black with Heidenhain's haematoxylin.

Oppel and Nicolas have described the occurrence of similar supernumerary sperm nuclei in Anguis fragilis, and Nicolas observed the tail-piece beside a number of them. In most details those described here agree with the descriptions given by Nicolas. Their long axes are parallel to the surface, none is quite so deep as the conjugating nuclei, and they contain a 'reticulum'.

The degree of polyspermy is evidently variable. Nicolas records the presence in eggs of Anguis fragilis of from one to forty-six. Oppel found up to five in the eggs examined by him. The smaller numbers occur more frequently. For the reason given by Nicolas, viz. that the sperm nuclei are all at the same stage of transformation, the relative number is evidently not affected by the time elapsing, since they must enter not successively, but more or less simultaneously.

Text-fig. 4.
A supernumerary sperm nucleus, showing middle-piece (M.P.) and sperm tail (T.). Nu, nucleus. YS, yolk-sphere. ×1,000.
Literature concerning the reptilian corpus luteum is rather scanty, and no account of the corpus luteum in Geckonidae has so far appeared. In 1893 Mingazzini (1893) published a paper on 'Corpi lutei veri e falsi dei Rettili', distinguishing corpora lutea and atretic follicles. He showed, principally in Seps chalcides, that the follicular epithelium is not expelled at ovulation, but remains within the follicle and takes part, together with fibroblasts from the theca, in the formation of the corpus luteum.

In 1903 Lucien described the formation of the corpus luteum in the viviparous lizards, Anguis fragilis and Seps chalcides, by the hypertrophy (but probably not mitotic division) of the follicular epithelium, and the invasion of the luteal tissue by connective tissue from the theca.

Hett, in 1924, gave a detailed account of the development of the corpus luteum from the follicular epithelium in the oviparous Lacerta agilis. Connective tissue, containing collagen fibres and supporting capillaries, was shown to penetrate the luteal tissue. The presence of a well-developed corpus luteum is therefore not dependent on viviparity.

Cunningham and Smart, in 1934, described corpora lutea in Lacerta viridis, Anguis fragilis, and Zootica vivipara in support of their theory that 'the true corpus luteum, i.e. the persistence and development of the follicular cells within the ruptured follicle, with associated changes in the cells of the theca interna, is the consequence and result of the internal development of the fertilized ovum in oviduct or uterus, or in the cavity of the ovary, and that in oviparous forms the ruptured follicle at once begins to undergo reduction and absorption'.

An examination of their material has shown, however, that in the case of the oviparous Lacerta viridis, they described and figured in their Text-fig. 3 and fig. 7, Pl. 14, a supposed corpus luteum which is none other than an atretic follicle, with thickened theca and a layer of giant cells derived from the follicular epithelium. It contains a central cavity in which are
yolk debris. They apparently failed to observe in the same ovary two well developed, typical corpora lutea containing strands of ingrown fibroblasts and blood-vessels. Their stage 3 is a fairly early one in the regression of the corpus luteum, marked by the enlargement of some nuclei preparatory to degeneration, as occurs in some other lizards and in Monotremes. Some sections of ovaries of Lacerta viridis belonging to Professor J. P. Hill show stages in the development of the corpus luteum by hypertrophy of the follicular epithelium, and confirm the fact that it is as well formed in this oviparous lizard as in viviparous ones.

Weekes, also in 1934, published a comparative account of corpora lutea in various oviparous and viviparous Australian scinks and in Lacerta vivipara. She found in all these lizards corpora lutea more or less penetrated by connective tissue from the theca, showing 'that luteal formation is to be also associated with oviparity'. The degree of penetration by fibroblasts has no relation to viviparity, since in three viviparous scinks this was only superficial, while in three others definite strands of connective tissue carry blood-vessels into the luteal tissue, and in one of these fibroblasts also penetrate between the luteal cells. Moreover, 'ingrowths of fibroblasts among luteal cells cannot be particularly associated with placentation, as it occurs in the oviparous lizards Amphibolurus muricatus and Lacerta agilis (Hett, 1924).

Since in Hoplodactylus only one egg is ovulated annually from each ovary only one corpus luteum develops in each. In the fresh ovary it appears in early stages as a creamy-white, flattened body, the opening through which the oocyte escaped being visible as a small, somewhat elongated depression stained with clotted blood. It is easily distinguishable from the more translucent white young oocytes, and forms rather more than half the volume of the ovary. At later stages its size is diminished and its surface becomes rounded. The shape of the corpus luteum in later stages varies considerably in individuals. The shape assumed is a matter of chance, being that which best fits the depression between the oocytes and leaves the surface of the ovary as regular as possible.
The history of the corpus luteum is described below under six arbitrary stages, numbered from 1–6.

Stage 1.—The youngest corpus luteum obtained relates to a uterine egg in which the male and female pronuclei are in apposition. It is flattened and measures 2.25 x 0.45 mm. in diameter. The egg ovulated measures 8 mm. in diameter. The luteal tissue, including the central cavity, is 1.28 x 0.45 mm. in diameter.

The theca is much thickened since ovulation, having increased from 0.05 mm. to 0.25 mm. (cf. fig. 7, Pl. 14; fig. 14, T., Pl. 16). The increase is due, at least partly, to shrinkage; but there may be some proliferation also. Weekes (1934) records the presence of mitoses in the theca of lizards at an earlier stage in the formation of the corpus luteum, and O'Donoghue (1916) observed it in the Marsupial Phascolarctos cinereus. No mitotic figures were observed here; but a few strands of spindle-shaped cells growing out from the damaged wall of the theca, near the opening, indicate the occurrence of mitotic or amitotic cell division.

In the theca of the ripe follicle the fibroblasts are concentrically arranged; but evidently as a result of the shrinkage following ovulation they are now radially arranged. The inner border of the theca is conspicuous owing to the presence of numerous deeply-staining fibroblast nuclei. Elastic fibres are particularly numerous here, and both nuclei and fibres are concentrically directed. Under low magnification this has the appearance of a basement membrane; but it is not a sharply defined layer (figs. 14 and 15, T., Pl. 16). It is probably derived from the membrana propria of the ripe follicle. Hett and Weekes distinguish as theca interna the inner part of the theca, which they find to contain fewer fibres than the rest. Evidently it is not comparable with the inner part of the theca in this case; and, since this part has nothing in common with the theca interna in mammalia, it is not called so.

Large blood-vessels run in the outer part of the theca, while small capillaries are very numerous in its inner border. Some of these project into the luteal tissue accompanied by fibroblasts from the theca (fig. 16, C., Pl. 17). Fibroblasts have also
begun to grow in strands into the luteal tissue from the whole inner surface of the theca (fig. 15, F.B., Pl. 16). These cells are derived not only from the inner border of the theca, but also from the tissue beneath, cells of which break through and irritate the inner border. Spaces occur between the ingrowing fibroblasts just inside the theca, and thus the luteal tissue in many parts is separated from the theca. The fibroblast nuclei often stain more deeply than the luteal nuclei and are smaller and elongated. Both elastic and collagen fibres penetrate the luteal tissue, and some reticular fibres also occur in it.

There is no definite evidence of the fate of the small patches of cells which form a doubtful theca interna in the ripe follicle. Here and there cells with small spherical nuclei, averaging 0.005 mm. in diameter, are situated singly or in small groups in the bases of the ingrowing strands of fibroblasts, which surround them. It is probable that these represent the cells of the theca interna, and they will be called so. They may have multiplied after ovulation, but are still not very frequent, and therefore cannot play an important part in the function of the corpus luteum.

The luteal tissue has almost obliterated the flattened cavity of the burst follicle, except opposite the opening (fig. 14, Pl. 16). The cells are radially arranged, as in the marsupial corpus luteum, and consequently the tissue appears to be composed of radiating strands of cells. The cytoplasm, which has increased in bulk since ovulation, is granular and stains well. There are a few intracellular spaces, and numerous radially compressed intercellular spaces (fig. 15, Pl. 16). The epithelial nuclei of the ripe follicle average 0.005 mm. in diameter, while the oval luteal nuclei average 0.008 mm. (fig. 16, L.C., Pl. 17). A few of these nuclei, irregularly distributed, stain deeply.

No mitoses were observed in the luteal cells; but division may take place earlier, as Weekes observed mitoses in an earlier stage in *Amphibolurus muricatus*. The arrangement of the cells suggests that multiplication has occurred. All trace of the folding of the follicular epithelium after ovulation, observed in a corresponding stage in the corpus luteum of another lizard by Weekes, has been obliterated.
The cavity of the corpus luteum contains degenerating blood corpuscles, some cell debris, and also occasional detached healthy-looking cells. Gatenby and Hill (1924), in their paper on the Monotreme corpus luteum, remark that these seem to be of no particular significance.

It is interesting that the ovum related to this corpus luteum is at exactly the same stage as those related to the earliest corpora lutea obtained by Lucien (1909), viz. at fertilization; but it has reached a stage of development considerably later than that of his. Lucien found the follicular epithelium 'plissé, revenu sur lui-même et décollé par endroits sur une étendue plus ou moins grande par des extravats sanguins'. The luteal cells described above have, however, altered considerably from the follicular cells of the ripe oocyte; and the thecal irruptions indicate that more time has elapsed between ovulation and fertilization in this case than in those of Lucien. This corpus luteum is also at a later stage than the early ones described by Weekes which were related to eggs already segmented. It may, perhaps, indicate that ovulation occurs independently of copulation and consequent fertilization; but it may be merely the result of some irregularity in behaviour.

Stage 2.—The next available stage of development of the corpus luteum is one where the related egg contains an embryo with approximately thirty somites. It is more or less spherical, measuring 1.44 mm. in diameter, while the luteal tissue measures 1.1 mm. The opening has narrowed considerably, and its margin is turned in and projects into the luteal tissue. The cavity is now filled with luteal tissue except for a small space inside the opening. The whole organ is much more compact than at stage 1, and stains more deeply. It has the appearance of an actively secreting body.

The theca averages 0.17 mm. in thickness (fig. 16, T., Pl. 17). The nuclei in it are smaller than before, and often irregular. The great majority stain intensely. The fibres have lost their radial arrangement. The inner border of the theca is still distinctive and has not altered particularly. Capillaries are very numerous; but they no longer project into the luteal tissue. Vacuoles occur frequently in their vicinity.
The theca interna cells have become more or less cubical and have granular cytoplasm (fig. 17, T.I., Pl. 17). Their nuclei are slightly larger than in stage 1.

A considerable change has taken place in the luteal tissue. The cells are more crowded and the nuclei are smaller and more variable in size. The latter often stain a homogeneous dark blue in haematoxylin; but others do not stain quite so deeply, while a few here and there stain lightly. Frequently a nucleus is indented by a vacuole, or sometimes by several minute ones about it. This is perhaps the most noticeable feature of the corpus luteum at this stage (fig. 17, Pl. 17). The vacuoles are occupied by a lipoid substance which blackens with osmium tetroxide (fig. 21, Pl. 19). It may contain secretions of physiological importance. The vacuoles are very numerous and measure up to 0.007 mm. in diameter. In addition to the definite vacuoles there are numerous irregular intercellular spaces, traversed by fibroblasts, which give the tissue the appearance of an irregular network.

The strands of fibroblasts growing in from the theca form, as it were, septa in the corpus luteum (figs. 16, 17, Pl. 17). The larger strands are surrounded by a kind of thin membrane of collagen, elastic, and even reticular fibres (fig. 18, Pl. 18). Fibroblasts from the strands ramify among the individual luteal cells and form an investment round them (fig. 17, Pl. 17). There is no connective tissue core, such as occurs in some lizards and Marsupials, in this corpus luteum. Blood-vessels are absent from the luteal tissue.

Stage 3.—Corpora lutea at this stage are related to eggs containing embryos 33 mm. in length from snout to tip of tail. The one described here is club-shaped in transverse section, though it appears rounded in situ in the ovary. It is 1.8 mm. in diameter and averages 0.6 mm. in thickness. The luteal tissue averages 1.7 × 0.5 mm. The opening is still obvious in sections, but has been filled up to the surface level with luteal tissue in which the inturned margin of the opening is embedded. The thecal tissue has not grown over the cicatrix.

The theca has become markedly thinner, averaging only 0.05 mm. in thickness. This reduction probable accounts for the
concentric arrangement of many of the fibroblasts, since they are somewhat stretched round the luteal tissue. The distinctive inner border of the theca has disappeared, owing, no doubt, to irruption by the ingrowing fibroblasts. The thecal nuclei are more or less flattened, and for the most part stain intensely; but, particularly at the inner side of the theca, many are undergoing chromatolysis. Capillaries in the theca are somewhat smaller and fewer than in earlier stages.

The theca interna cells remain few in number but the nuclei have increased in size, averaging 0.009 mm. in diameter. They are often bluntly triangular or oval in shape. The enlargement may be preparatory to degeneration, or they may play some part in the regression of the corpus luteum; but their function is obscure. It would appear that they are too few in number to effect any extensive changes. A coagulum is present among the fibroblasts just inside the theca where the theca interna cells are situated. It may be a product of cytolysis in the theca, for here and there small clumps of degenerating thecal cells project slightly into the spaces among the fibroblasts. Weekes (1934) describes a similar coagulum in the skink Lygosoma quoyi. A few eosinophil and polymorphonuclear leucocytes have appeared in the corpus luteum, principally in these parts.

The luteal cytoplasm appears denser than in stage 2. Spherical vacuoles containing a lipoid substance are still numerous; but a coagulum has appeared in many of the intercellular spaces. This is finely granular and differs from that among the fibroblasts. The luteal nuclei are less frequently indented by vacuoles at this stage. A few are pyknotic, and many stain rather deeply. Here and there nuclei occur which have regained their size in stage 1; but the majority are only about half that size. There are no capillaries in the luteal tissue.

Secretion is clearly diminished at this stage, and part of the lipoid secretion present before has been removed. The reduction in the blood supply of the theca is an index of the reduced activity of the organ.

Stage 4.—This stage is just a little later than the last, but the corpus luteum shows further regression. It is oval in transverse section, rounded in surface view, and considerably smaller
than stage 3, measuring 0.8 × 0.54 mm. in diameter, while the luteal tissue measures 0.7 × 0.45 mm. The opening in the theca is not appreciably smaller than before, but is plugged with luteal cells now bounded externally by a single layer of fibroblasts which appear to be derived from those in the luteal tissue rather than directly from the theca (fig. 20, O.P., Pl. 18).

The theca averages 0.05 mm. in thickness, and therefore is not much reduced since stage 3, except that it has contracted round the diminished luteal tissue without increasing in thickness (fig. 20, T., Pl. 18). A corpus luteum intermediate between this one and stage 3 measures 1.08 × 0.72 mm. in diameter, and the theca is also 0.05 mm. in thickness. The contraction of the theca probably accounts for the fibres again showing irregular arrangement. The nuclei are, as a result, less flattened than in stage 3. Their size is variable: some show an increase and stain lightly. The cytoplasm stains well, partly due, perhaps, to the presence in it of the coagulum observed inside it at the last stage. Eosinophil leucocytes are more frequent than before, both in the thecal and luteal tissues. They are particularly numerous among the fibroblasts at the bases of the septa. The large theca interna nuclei here stain only lightly (fig. 19, T.I., Pl. 18). A further reduction has occurred in the size and number of the capillaries in the theca.

The luteal tissue resembles that of stage 3. The reduction of the intercellular spaces causes it to appear a little denser. The arrangement of the narrow spaces causes the cells to appear separated into strands. Occasional nuclei are irregular, shrunken, and stain lightly. The most important change at this stage is the appearance of a small ragged space in the middle of the luteal tissue (fig. 20, Pl. 19). This is a result of degeneration of the cells, some of which are present in the space. Degeneration, therefore, sets in first at the centre, as in other Reptiles (Oppel, 1892) and in Monotremes (Hill and Gatenby, 1926).

Stage 5.—This stage is from a female with intra-uterine eggs containing young very shortly before birth, measuring about 40 mm. from snout to tip of tail. In this case the corpus luteum is kidney shaped, both in surface view and transverse section, and measures approximately 0.8 × 0.5 mm. in diameter
while the luteal tissue is $0.7 \times 0.4$ mm. in diameter. The thecal tissue has not grown over the cicatrix, the thecal opening being plugged only by luteal tissue.

The theca is further reduced, averaging $0.04$ mm. in thickness. The fibroblasts are again directed more or less concentrically (fig. 22, $T.$, Pl. 19). The tissue stains deeply, probably because it is permeated by an eosinophil coagulum described in stages 3 and 4. There are often spaces between the fibroblasts, particularly in the outer part of the theca. Capillaries are small and few. Many of the nuclei are undergoing chromatolysis.

The number of fibroblasts among the luteal cells has increased considerably. The luteal tissue is very vacuolated, both the size (up to $0.012$ mm.) and the number of the vacuoles having increased, giving the tissue the appearance of a network. Many of the nuclei are irregular or even pycnotic. Chromatolysis is occurring in others. The irregular ragged space near the middle of the tissue is still not large, but this is probably on account of the shrinkage of the organ around it.

Stage 6.—The final stage available was obtained from a female some days after the birth of the young. Owing to individual variations in the breeding season, the time elapsing since parturition cannot be given more exactly. The corpus luteum measures $0.33$ mm. in diameter. Although small, it is easily distinguishable among the oocytes on account of its characteristic deep cream colour.

The boundary between the theca and luteal tissue is very indefinite, owing to the general degeneration and the growth of fibroblasts into the luteal tissue (fig. 23, Pl. 19). Where it is distinguishable, the thickness of the theca is about $0.02$ mm. Many of the nuclei have disappeared, and the fibres are apparently degenerating.

Many of the luteal nuclei have also disappeared, so that only comparatively few remain (fig. 23, Pl. 19). A few of these are undergoing chromatolysis, while others are enlarged to twice their former size and stain lightly. Still others are a little shrunken or irregular. Large vacuoles have appeared in the cytoplasm, which stains very lightly and is markedly alveolar.
in character. Cell limits are not distinguishable. The fibroblasts have largely disappeared from among the luteal cells, but some of the larger strands remain. A coagulum, which stains like collagen with Crossmann-Mallory triple stain and may be derived from the degeneration of the fibres, occurs both in the theca and in parts of the luteal tissue. There is no longer a space in the middle of the luteal tissue.

Owing to the degenerate state of the theca the point of rupture at ovulation is no longer distinguishable. It is evident from the comparatively short time elapsing between stages 5 and 6 that rapid regression is occurring, and the corpus luteum will shortly have disappeared completely.

DISCUSSION.

The corpora lutea in this lizard and in *Lacerta vivipara* (Weekes, 1934) are the only ones so far described in reptiles where fibroblasts penetrate between individual lutein cells as well as form septa. In *Lacerta vivipara*, however, blood-vessels grow in with the connective tissue. In the corpora lutea of the viviparous skinks *Lygosoma quoyi*, *Lygosoma quadridigitatum*, and *Egernia whitei*, there is also no penetration by blood-vessels; but ‘there are only superficial ingrowths of fibroblasts among the luteal cells and consequently there are no blood-vessels among the luteal cells’. This difference indicates, however, that blood-vessels do not necessarily accompany the ingrowth of connective tissue septa. The penetration of fibroblasts is greater in *Hoplodactylus* than in *Lacerta viridis*, where there are blood-vessels in the luteal tissue.

Another variation from most reptilian corpora lutea described, though probably of no significance, is the persistence of the opening in the follicle which becomes plugged only with luteal tissue, and is not grown over by the theca. This is interesting as the opening in Monotremes and Eutheria also becomes plugged with luteal cells and covered by fibroblasts from the septa.

Hett and Weekes found that the inner part of the theca where the capillaries lie contains fewer fibres than the remainder,
and designate it ‘theca interna’. This distinction does not occur in *Hoplodactylus*; and, since this use of the term does not correspond with that in mammals, it was thought well to avoid it in reference to the inner part of the theca, and to apply it, as in mammals, to a very discontinuous layer of cells without fibres just outside the membrana propria of the ripe follicle. This designation brings the structure of the corpus luteum into line with that of the Monotreme (Hill and Gatenby, 1926), where strikingly similar septa are formed by the ingrowth of fibroblasts from the theca externa. In the Monotreme they irrupt the theca interna, just as they pass between the theca interna cells where these are present in *Hoplodactylus*. In both cases the theca externa provides, in addition, fibroblasts which ramify among the individual lutein cells. The latter have a similar derivation, resulting from the hypertrophy and possibly, at the earliest stages, the mitotic division of the follicular epithelium. Minor points of similarity are the thinning of the theca as development proceeds, the commencement of regression at the centre first, and its accompaniment by the appearance of a coagulum. An important point of difference is the absence of any lipoid secretion in the Monotreme. In this respect *Hoplodactylus* agrees with the Eutheria.

With regard to the rudimentary state of the theca interna, the corpus luteum of *Hoplodactylus* resembles more closely that of the Marsupials (O’Donoghue, 1916) than the Monotremes; but O’Donoghue states that cells of both theca interna and theca externa grow in to form septa and a central core. The structure and appearance of the corpora lutea in the two cases are, however, strikingly similar, although this lizard lacks blood-vessels in the luteal tissue, and a core of connective tissue.

From comparison it appears that notwithstanding the failure of blood-vessels to penetrate the luteal tissue, the corpus luteum in *Hoplodactylus* reaches a higher stage of development than do those where the penetration of fibroblasts is superficial, or where they are not interstitial but confined to septa. Secretions can easily be passed in the intercellular spaces, which, owing to the strand-like arrangement of the
luteal cells, resemble irregular intercellular channels, to the richly vascularized inner border of the theca.

At present it is quite impossible to predict the structure of the reptilian corpus luteum from a knowledge of that of another member of the genus, from the behaviour at the breeding season (whether oviparous or viviparous), or from the structure of the placenta. The variations of structure which occur in this lizard do not clarify correlation. It would appear that the corpus luteum in reptiles has arisen independently in the various species, and independently of the presence or absence of placentation. Since corpora lutea are formed in oviparous reptiles they may have appeared before the placenta, and later have acquired a function connected with placentation.

No work has yet been done on the physiology or nature of the secretions of the reptilian corpus luteum. It is, perhaps, not without significance that Weekes found that 'in the viviparous species atresia begins early in the gestation period and continues until the birth of the young'; but in *Hoplodactylus* no atretic follicles have been found in the ovaries of pregnant females. Instead, the ova do not enlarge but remain very small during the whole of the gestation period, i.e. during the life of the corpus luteum. In a paper to appear shortly (Boyd, 'Proc. Zool. Soc.'), a description of placental changes in a non-pregnant uterus of a female *Hoplodactylus* in which the other uterus is pregnant indicates that placentation in reptiles is under the influence of hormones, one or more of which may originate from the corpus luteum.

**Summary.**

The structure of the ovary, including stages in the ripening of the oocytes, is described. A prolonged diplotene stage with 'lamp-brush' chromosomes is shown to occur in reptiles, as in other classes of vertebrates with large yolky eggs.

The striated layer of the egg membrane is shown to be composed of the same cuticular substance as the zona pellucida. A follicular epithelium composed of three types of cells, later reduced to a single layer of small cells, agreeing with Loyez's observations, is described.
A discontinuous theca interna, comparable with that of mammalia, is noted outside the membrana propria of the nearly ripe oocyte.

A thin, soft, fibrous shell membrane is formed round the uterine egg and polyspermy occurs. The latebra, and the male and female pronuclei in apposition, are described.

The corpus luteum is shown to consist of luteal cells invested by fibroblasts from the theca externa. Septa of fibroblasts are also present, but no blood-vessels. The theca is rich in capillaries. The theca interna plays no part in the development of the corpus luteum. A lipoid secretion, which may be of physiological importance, is formed in it. It is compared with that in Monotrems and Marsupials.

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EXPLANATION OF PLATES 13–19.

LETTERING.

B.V., blood-vessel; C., capillary; Chr., chromosomes; Cy., cytoplasm;
E.F., elastic fibres; F., fat-globules; F.B., fibroblast; F.C., follicle cells;
G.E., germinal epithelium; I.F., intermediate follicle cells; L., lipid
substance; L.a., latebra; L.B.C., lamp-brush chromosomes; L.C., lutal
cell; L.F., large follicle cell; L.N., latebral neck; M.P., membrana própia;
M.T., opening of tubular prolongation of large follicle cell through zona;
Nl., nucleolus; Nu., nucleus; O.P., opening in theca; P., pigment; S.,
fibroblast septum; S.F., small follicle cell; S.M., shell membrane; S.Z.,
striate zone; T., theca; T.F., tubular prolongation of large follicle cell;
T.I., theca interna cell; V., vacuole; V.M., vitelline membrane; Y.Nu.,
yolk-nucleus; Y.S., yolk-sphere; Z., zona pellucida.
EXPLANATION OF PLATES 13-19.

PLATE 13.

Fig. 1.—Nucleus of 0·015-mm. oocyte in the germinal bed, showing the blob-like chromosomes. ×950.

Fig. 2.—Nucleus of 0·025-mm. oocyte (left) in early diplotene stage, and of 0·09-mm. oocyte (right), showing vacuolated nucleolus and 'lampbrush' chromosomes. ×780.

Fig. 3.—Nucleus of 0·175-mm. oocyte, showing lamp-brush chromosomes and large nucleoli. ×630.

Fig. 4.—Nucleus of 0·6-mm. oocyte, showing lamp-brush chromosomes condensed to beaded threads, and numerous small nucleoli. ×560.

PLATE 14.

Fig. 5.—Follicle cells and zona of a 1-mm. oocyte, showing the perforation of the zona by tubes from the large follicle cells. ×825.

Fig. 6.—Part of the follicle and periphery of an 0·8-mm. oocyte, fixed in Maximow's fluid, to show fat. ×630.

Fig. 7.—A portion of the theca, follicle, and zona of a 6-mm. oocyte, showing theca interna cells, thin zona pellucida, and thick striated layer. ×860.

Fig. 8.—A supernumerary sperm nucleus in the same uterine ovum. ×1,330.

Fig. 9.—The yolk-nucleus of a 1·1-mm. oocyte fixed in Maximow's fluid. ×710.

PLATE 15.

Fig. 10.—Membranes of the uterine ovum. ×1,100.

Fig. 11.—The male and female pronuclei in a uterine ovum. ×1,330.

Fig. 12.—Follicle cells of a 1·25-mm. oocyte. ×825.

Fig. 13.—The latebra in a uterine ovum. The winding latebral neck is cut in three places. ×95.

PLATE 16.

Fig. 14.—A recently burst follicle, from which the uterine ovum described was liberated, in which a corpus luteum is developing. ×45.

Fig. 15.—A portion of fig. 14, more highly magnified, showing fibroblasts among the luteal cells. ×825.

PLATE 17.

Fig. 16.—Portion of an older corpus luteum (stage 2) showing ingrowing septa of fibroblasts. ×190.

Fig. 17.—Portion of fig. 16 under a higher power. ×710.

Fig. 18.—A slightly older corpus luteum stained to show elastic fibres. ×80.
PLATE 18.

Fig. 19.—Portion of fig. 20 more highly magnified to show theca interna cells at the bases of the fibroblast septa. $\times 710$.

Fig. 20.—A corpus luteum in an early stage of regression, showing the presence of an irregular central cavity. The opening in the theca persists, plugged only by luteal tissue. $\times 120$.

PLATE 19.

Fig. 21.—Portion of a corpus luteum of about the same age as that in fig. 18, fixed in Maximow's fluid to show the lipoid secretion. $\times 320$.

Fig. 22.—Portion of an older corpus luteum, showing the reduction of the theca. $\times 300$.

Fig. 23.—Late stage in the regression of the corpus luteum, taken from a parent shortly after the birth of the young. $\times 210$. 
Fig. 1

Fig. 2

Fig. 3

Fig. 4

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Fig. 14

Fig. 15

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Fig. 17

Fig. 16

Fig. 18

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