STUDIES ON THE CORPUS LUTEUM IN *ENHYDRINA SCHISTOSA* (DAUDIN) AND *HYDROPHIS CYANOCINCTUS* (DAUDIN) OF THE MADRAS COAST

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Introduction

The corpus luteum of Reptiles has attracted considerable attention and lizards have been the favourite objects of study. In this paper are described, for the first time, the stages of development of the corpus luteum and its degeneration in the viviparous sea-snakes *Enhydrina schistosa* and *Hydrophis cyanocinctus* occurring on the Madras coast.
As early as 1892, Strahl worked out the reconstruction of the ruptured ovarian follicle of *Lacerta agilis* and in 1893 Mingazzini published a paper on “Corpi lutei veri et falsi dei Rettili”. Mingazzini working on *Seps chalcides* observes that the follicular epithelium is not discharged at ovulation but undergoes certain changes and takes part in the formation of the corpus luteum. Later on in 1903, Lucien studied the formation of the corpus luteum in the viviparous lizards, *Anguis fragilis* and *Seps chalcides* and published a ‘note préliminaire sur les premières phases de la formation des corps jaunes chez certaines Reptiles’ in which he declared his belief that the corpus luteum is formed by the hypertrophy of the follicular epithelium, unaccompanied by mitotic division, but with invasion of the connective tissue theca. Hett’s work in 1924 on the corpus luteum in *Lacerta agilis* deals with the structure and development of the follicle and the formation of the corpus luteum after the rupture and escape of the egg. In 1933 Hett published an important review, under the title “Vergleichende Anatomie der Corpora lutea”. A comparative study of the corpus luteum in certain oviparous and viviparous lizards by Weekes in 1934 is a more recent contribution. This author gives a description of the histology and development of the corpus luteum in *Amphibolurus muricatus* and *Lygosoma* (*Hinulia*) *quoyi* and a general comparative study of the corpus luteum in the Australian viviparous lizards *Egernia whitei*, *Lygosoma* (*Hemiergis*) *quadridigitatum*, *Egernia cunninghami*, *Lygosoma* (*Liolepisma*) *weekesae*, *Lygosoma* (*Liolepisma*) *entrecasteauxi* and the European lizard *Lacerta vivipara*. In the same year Cunningham and Smart published a paper on “The structure and origin of the corpora lutea in lower vertebrates”. These authors hold that the formation of the corpus luteum occurs only in viviparous forms and that in oviparous forms the ruptured follicle immediately undergoes degeneration. Boyd’s (1940) account of the formation of the corpus luteum in *Hoplodactylus maculatus* is another useful contribution.

**PART I**

**ENHYDRINA SCHISTOSA (DAUDIN)**

*Material and Methods*

Six pregnant snakes containing corpora lutea in various stages of development and one non-pregnant specimen containing the largest egg noted and ready for ovulation, form the material for this study. The specimens were collected from November to December, 1940. The snakes were chloroformed and the ovaries quickly dissected out as soon as the animals were dead. For general cytological details Bouin’s and Carnoy’s fluids were
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used and both gave good results. The material was also fixed in a variety of fluids such as Champy, Flemming without acetic, Regaud, corrosive-sublimate and 5% formalin. On account of the thick external coverings of the oocyte and the corpus luteum, fixation and subsequent treatment have to be longer than usual to obtain satisfactory results. Sections 4 to 7 μ in thickness were taken. Iron haematoxylin was used after all fixatives and as counterstains Eosin or Van Gieson’s. Mallory’s triple stain was also used.

Both Enhydrina schistosa and Hydrophis cyanocinctus are viviparous species the young of which are born without egg membranes. Both have a highly specialised allanto-placenta (Kasturirangan, 1941 unpublished). The facts observed indicate that the breeding season of Enhydrina schistosa probably extends from November to January. Both the ovaries are functional at the same time. The non-pregnant specimen obtained towards the end of November was found to contain the largest egg examined measuring about 70 mm. in length. Late in the same month ruptured follicles were first observed in the gravid specimens. Between November and December almost all the females examined were pregnant and the ovaries contained corpora lutea in various stages of development. In each ovary, one to three corpora lutea were commonly found. The corpora lutea in the ovary correspond in number to the embryos in each uterus.

The Structure of the Unruptured Follicle

The earliest stage of the oocytes obtained shows the round cords of epithelial cells, the egg tubes of ‘Pflüger’, growing into the stroma of the ovary where they ramify in complicated anastomoses. The cords enlarge towards the caudal region and become broken up into nests of germinal epithelial cells each of which may be taken to represent a Graffian follicle. At first it is not possible to differentiate the future ova from the cells destined to form the follicular cells. The egg cells become surrounded by a regular layer of small cells representing the follicular epithelium.

The smallest oocytes found in the ovary are situated just beneath the germinal epithelium and are surrounded by a few flattened follicle cells—the primordia of the membrana granulosa. The follicle cells have little cytoplasm but prominent well-developed nuclei containing chromatin reticulum. Slightly larger oocytes are surrounded by a single layer of more or less cubical epithelial cells. The egg now acquires a sheath of undifferentiated connective tissue cells and fibres. At this stage there is no distinction between theca interna and theca externa.

The fairly mature egg, oval in shape, projects slightly from the surface of the ovary. It is invested by the egg membranes—the vitelline membrane
and the zona radiata the latter being thicker and both are closely attached together. In the mature follicle the single layered follicular epithelium has become many layered. Two kinds of cells can be distinguished (Ph.M. 1). One set consists of large cells with massive granular cytoplasm each with a prominent nucleus and nucleolus and the chromatin in the form of a few more or less large chromatin granules arranged in a reticulate manner. Intercalated among these large cells are smaller cells with little cytoplasm and with spherical nuclei. A nucleolus can be distinguished here also but the chromatin reticulum is not clearly marked. These small cells form an outer layer next to the membrane propria and also extend between the large specialised cells and lie near the zona radiata. The presence of the remarkable large cells—a normal feature of the reptilian follicular epithelium—is interesting as it distinguishes the reptiles from the birds and mammals where the constituent cells are all of one kind. It may be recalled that there is the same difference between the cells of the follicular epithelium in the Raidæ (Samuel, 1943).

The large cells have probably something to do with the nutrition of the egg. Wallace (1904) distinguishes the follicular epithelial cells of *Chimæra* into large "nutritive cells and small indifferent cells" and states "the nutritive cells degenerate and disappear before the maturation of the egg".

Between the follicular epithelium and the connective tissue sheath lies the membrana propria, distinct from the theca folliculi but closely adherent to the follicular epithelium and consisting of a single layer of elongated cells with deeply staining nuclei.

The connective tissue sheath surrounding the follicular epithelium is much more developed, but the distinction between the theca interna and theca externa is not clear though the inner zone contains more of cellular elements (Ph.M. 1). The cells of this zone have spindle-shaped nuclei with their long axes parallel to the follicular epithelium. Minute blood-vessels can be discerned, with some difficulty, in the outer region of the connective tissue sheath.

The fully mature follicle is oval in shape and projects out from the surface of the ovary. The comparatively large size of the egg in the ovary just before ovulation is of interest in connection with the changes which occur after ovulation. The structure of the follicular wall is seen in Fig. 2 and Ph.M. 2. The follicular wall of the egg has undergone considerable stretching as a result of which the membrana propria is no longer seen as a distinct layer. Observations made on the follicular epithelium of the fully developed egg support the statement of Wallace (1904) regarding the
degeneration of the large nutritive cells in Chimæra. The large cells have undergone considerable reduction and degeneration. The chromatin of the nuclei clump together accompanied by the complete degeneration and disappearance of the nuclear membrane, cell-cytoplasm and cell-membrane (Ph.M. 2). Leucocytes, probably phagocytic in nature, are of common occurrence. Thus many of the cells break down and disappear and finally in the ruptured follicle there is absolutely no trace of the large cells.

The theca folliculi, surrounding the follicular epithelium, has considerably increased in thickness and is arranged in several layers (Fig. 2 and Ph. M. 2). This layer consists of connective tissue cells and fibres, but the parallel arrangement of the fibres is no longer evident as in the previous stage. The cells have oval deeply staining nuclei. Although the theca folliculi is greatly developed at this stage the theca interna does not exist as a separate layer. But the elements that would ultimately form it can be distinguished in the vicinity of the follicular epithelium as a narrow layer of connective tissue cells in a loosely arranged network of fibres. In certain regions the theca interna cells and the connective tissue fibres form a more or less definite, if somewhat irregular, layer within the more fibrous outer layer. Immediately outside the theca interna, the connective tissue itself is specially developed into closely approximated fibrous bands with a few cells in the interstices thereof, which later on form the theca externa. There is a remarkable increase in the number and size of the blood vessels in this region just before ovulation.

Corpus luteum

Stage I

The uterine eggs corresponding to the corpora lutea in the ovary were in the early blastoderm stage, and all the three eggs present were of the same size and stage of development. It seems that the eggs should have been liberated recently, possibly not more than 3 or 4 days prior to capture of the specimen.

The corpora lutea in the earliest stage are very conspicuous objects by reason of their size (25 mm. in length). They project from the ovary so that the remaining eggs which await a long period of growth and maturation corresponding to the duration of the gestation period appear dwarfs in comparison. All the corpora lutea from this ovary are similar in size and external appearance, and microscopic examination reveals identical cytological features. They are oval bodies situated in the mid-region of the ovary and pinkish white in colour without any trace of the yellow pigment, which is so characteristic of the corpus luteum in later stages. Figure 1 shows the corpus luteum with the ruptured opening of the follicle appearing as
a long slit with its shrunken edges dipping in. Easily visible blood vessels in the sheath tissue vascularize the burst follicle.

Figs. 1–5.—Fig. 1. The corpus luteum, Stage I, about three times natural size. Fig. 2. T. S. of the wall of the fully mature egg showing the follicular epithelium, theca interna and theca externa. ×120. Fig. 3. T.S. of the corpus luteum, Stage I, to show the hypertrophied follicular epithelial luteal cells, the well-defined theca interna and the theca externa carrying large blood vessels. ×80. Fig. 4. T.S. of the corpus luteum, Stage II, showing the increase in thickness of luteal tissue and the distinction between the theca compacta and theca spongiosa. ×80. Fig. 5. T.S. of the corpus luteum, Stage III, showing the change in the three layers, and ingrowth of connective tissue elements into the luteal tissue. ×80.
Photomicrograph 3 shows a low power view of a transverse section passing through the ruptured opening of the corpus luteum at this stage. When the follicle bursts and collapses, its walls are brought together so that the central cavity the 'antrum folliculi' is seen as a narrow slit in the centre which communicates to the outside through the opening. The ruptured opening is comparatively large, but gradually becomes smaller and finally closes in the later stages. The central lumen is occupied by a faintly staining coagulum intermingled with a few blood corpuscles and cells. These cells are identical with those forming the follicular epithelium. They are evidently detached from the follicular epithelium, owing to the pressure caused by the rupture and contraction of the wall of the follicle.

A portion of a transverse section of the corpus luteum is illustrated in Ph.M. 4. Owing to contraction, the follicular epithelium lining the lumen becomes folded and it varies from 4 to 10 cells in thickness (Fig. 3 and Ph.M. 4). It consists of large deeply staining luteal cells with characteristic spherical nuclei. The cells vary in shape from spherical to polygonal.

The cytoplasm is delicate, finely granular and the cells have definite cell limits. The nucleus stains deeply and possesses a large nucleolus and a well-marked reticulum of deeply staining chromatin granules. It is noteworthy that in the corpus luteum, in striking contrast to the unruptured follicle, we find only one kind of cell—the larger cells having degenerated and absorbed. The corpus luteum at this stage is actually a little smaller than half the size of the ripe egg, consequent on the rupture and escape of the egg—a common phenomenon in certain mammals. The follicular epithelial cells show hypertrophy which is quite evident from the larger size of the cells, in contrast to those of the unruptured follicle. There is an increase in the thickness of the follicular epithelium. This is partly due to the crowding of the cells brought about by the lateral contraction of the follicle and a simultaneous hypertrophy of the cells of the follicular layer. The presence of a few scattered and free blood corpuscles among the differentiating luteal cells is a feature noticed in this early stage. These corpuscles are derived from the vessels of the theca, the walls of which have broken down as a result of the rupture of the follicular wall. This intra-follicular haemorrhage is a characteristic feature of the higher mammals as described by Marshall (1904) in sheep, etc.

The Theca interna.—The theca folliculi plays a very prominent part in the formation of the early corpus luteum though it undergoes gradual reduction in the later stages. The connective tissue sheath as shown in Fig. 3 and Ph.M. 4 in contrast to its condition in the unruptured follicle has greatly thickened. There is now a clear distinction between the
theca interna and theca externa. Immediately after ovulation the theca interna becomes organised into a layer of connective tissue cells and fibres which lie just below the follicular epithelium (Fig. 3 and Ph.M. 4). The theca interna is comparatively thinner than the fibrous theca externa and follows the wavy contour of the follicular epithelium and has a varying thickness of 2 to 5 cells. The nuclei of the theca interna cells vary in shape and size and a few of them approximate very nearly in size to the nuclei of the follicular epithelial cells from which they can be clearly distinguished. The nuclei are deeply staining and have a distinct nuclear membrane. They contain a large number of intensely staining chromatin granules. The nucleoli are not clearly distinguishable from the chromatin granules in all the cells, as in the case of the follicular epithelial cells. The cytoplasm is faintly vacuolated. Hill and Gatenby (1926) attribute a definite secretory function to the cells of the theca interna which are most active in the earlier stages. A comparison with the unruptured follicle shows a definite increase in the number of the cells. This increase may be due to karyokinetic division though the process has not been observed by me. But Hill and Gatenby (1926) describe and figure a few definite mitotic divisions in the theca interna of the early corpus luteum of Platypus. Hett (1924) observes mitotic divisions of the cells of the theca interna in Lacerta agilis.

The Theca externa.—The theca externa is more fibrous and very much thicker than the theca interna (Fig. 3 and Ph.M. 4). The outer portion of the theca externa is traversed by a number of blood vessels and is distinguished as the theca spongiosa. The inner portion which is more compact, thinner and usually free from any blood vessels is called the theca compacta. In the theca externa the connective tissue cells are very rare but is rich in connective tissue fibres and loosely arranged fibro-blastic cells with deeply staining spindle-shaped nuclei. It has already been noticed that the vascularisation of the follicle wall commences immediately prior to ovulation. After rupture there is a greater increase in the number of the large blood vessels in the theca spongiosa. Some of them can be seen penetrating further towards the interior of the follicle. Extravasated blood corpuscles are found not only near the point of rupture of the follicle but also in the theca interna as well as in the theca externa. These blood corpuscles are apparently derived from the large blood vessels of the theca spongiosa the walls of which have broken down.

Stage II

At this stage the blastoderm is well formed and there is a well marked network of blood vessels on the blastoderm. The corpus luteum
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Corresponding to this stage measures 15 mm. in length. It has undergone marked changes. It is a more or less oval body coloured light yellow.

The central lumen is very much reduced as a result of ingrowths of luteal cells partially filling the cavity which however still communicates with the exterior through a minute opening representing the original rupture.

The Luteal tissue.—The follicular epithelial (luteal) cells now show considerable hypertrophy and present a distinct increase as compared with those of the previous stage. Mitosis has not been noticed by me but has been described in the follicular epithelial cells among the early stages of the formation of the corpus luteum in the Marsupials, Didelphys aurita (O'Donoghue, 1916), in Sheep (Marshall, 1904) and in the lizard Amphibolurus muricatus (Weekes, 1934). The last mentioned author states that follicular epithelial cells divide both mitotically and amitotically but that the mitotic figures are rare. Evidence of a similar multiplication by mitosis has been brought forward by O'Donoghue (1914) in the case of Perameles obesula and Perameles nasuta. None of them mention the phenomenon as very common. It is therefore obvious that the increase in the luteal tissue is to be attributed more to the hypertrophy of the already existing cells than to any considerable multiplication as a result of mitosis.

The luteal cells are the largest elements in the corpus luteum, varying in shape from spherical to polygonal cells. The majority of the cells have the characteristic vesicular nuclei, with a large irregular karyosome and a dense network of chromatin granules. Some of the nuclei are oval in shape. As the luteal condition is assumed the nucleoli become more and more a distinct feature of the nuclei of the follicular cells. There is a slight variation in the staining capacity among the nuclei of these cells, some of them taking on a lighter stain while others stain deeply with iron hæmatoxylin. The cytoplasm is very finely granular and stains moderately. The perinuclear cytoplasm generally appears to be denser and more deeply staining than the ground cytoplasm surrounding it.

The Theca interna.—Both the theca interna and externa are thinner than in the previous stage and the inner surface of the former has a more even contour (Fig. 4). There is a well-marked difference between the cells of the theca and follicular cells in this stage. The cytoplasm of some of the theca cells is distinctly vacuolated and cell boundaries are not readily distinguishable. The nuclei become more variable in regard to size and shape. Some of them are larger than some of the follicular cell nuclei. The nucleus and the chromatin network are distinct and intensely staining.
Ingrowths resembling little bud-like projections arise from the inner side of the theca interna. It may be stated that the majority of cells come to lie near the follicular epithelium and are most numerous at the base of ingrowths.

The Theca externa.—Though the theca externa is thinner and more fibrous than in the previous stage, the distinction between the theca compacta and theca spongiosa is much more marked than in the earlier stage (Fig. 4). The former is now separate and is formed of parallel layers of closely arranged fibroblastic cells with oval or elongated nuclei of different sizes, varying in thickness from 3 to 5 cells. The large blood vessels are concentrated in the theca spongiosa which is composed of irregularly arranged fibroblastic cells with deeply staining nuclei and connective tissue fibres. There is no appreciable increase in the number of blood vessels.

Stage III

The uterine egg corresponding to the corpus luteum of this stage is approximately in the same stage of development as in the preceding one.

The corpus luteum though of the same shape and size as in the previous stage shows further histological changes. It is almost filled with fully developed luteal cells, except for a small lumen which is seen as a narrow compressed space in the centre in transverse sections passing through the middle. The external opening still persists (Ph.M. 5).

The Luteal tissue.—A portion of a transverse section of the entire corpus luteum is shown in Ph.M. 5. Fig. 5 and Ph.M. 6 show the structure of the wall of the corpus luteum.

The luteal cells with their prominent vesicular nuclei attract attention. They are the largest elements in the corpus luteum and have now attained a relatively large size showing an active glandular appearance (Ph.M. 6). The luteal cells have a distinct wall at this stage and most of them have assumed a spherical shape.

The nuclei are of maximum size, spherical, and apparently in a highly active condition. There is, in each nucleus, a typically large irregular karyosome besides a few smaller nucleoli with all of which a well-marked reticulum studded with deeply staining chromatin granules is connected. The same variation in staining as occurs among the nuclei of the previous stage is noticed in a slightly more marked degree. With the assumption of fully developed luteal condition a regular system of minute vacuoles appears close to the nucleus, in the cytoplasm. They are very few and small at this stage.
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**The Theca interna.**—A close study shows that the connective tissue ingrowths from the theca interna initiated as bud-like projections in the previous stage, are carried further and are in the process of penetration between the luteal cells (Fig. 5, Ph.M. 6). The theca interna cells differ in no essential features from those of the preceding stage but are slightly more vacuolated. Several of them show a clear space between the nucleus and the cell membrane. Inter-cellular spaces begin to appear in the layer. A few scattered blood-corpuscles are present.

**The Theca externa.**—The theca externa is very much of the same thickness as in the previous stage. The nuclei of the fibroblastic cells however appear to be distinctly larger. The fibroblastic cells and fibres of the theca compacta still maintain their parallel arrangement. There is a slight decrease in the vascularisation of the theca and the large blood vessels are confined to the theca spongiosa.

**Stage IV**

The corpus luteum is slightly reduced in size measuring 12 mm. while the embryo in the uterus has become more advanced measuring approximately 44 mm., in length. The external opening is closed and is marked by a longitudinal depression filled by a mass of yellow substance.

A transverse section of this stage presents a still later phase of development. The central lumen still persists. A plug of luteal tissue projecting slightly to the outside now closes the external opening (Ph.M. 7). Such a protrusion of the luteal cells has been described by many authors in higher mammals, in rabbit by Sobotta (1897), in bat (*Vesperugo noctula*) by Van der Stricht (1912), in pig and cow by Corner (1919) and in *Platypus* by Hill and Gatenby (1926). Corner, in his account of a corresponding protrusion in the pig, says that the hypertrophy of the luteal cells is the cause of the protrusion of a considerable portion of the luteal tissue into the wall of the follicle weakened by the rupture, while in the swine the luteal plug is not of common occurrence because of the distention the whole wall undergoes. Corner further mentions the constant occurrence of the plug of luteal tissue in the corpus luteum of the cow which lasts throughout pregnancy. The protruding plug of luteal cells described in this stage corresponds to that of the above-mentioned authors and to the ‘Ppropt’ of German authors and that of Van der Stricht (1912) who terms it ‘le bouchon épithéial obturator’. But unlike in the cow, this plug becomes soon covered over by the connective tissue sheath in later stages. It may be stated that a similar plug of luteal tissue is not mentioned in the ruptured follicles of reptiles except in *Hoplodactylus* by Boyd (1940). Hett (1924) in his account of the
development and histology of the corpus luteum in *Lacerta agilis* and Weekes (1934), who deals with the corpus luteum in the oviparous *Amphibolurus muricatus* and the viviparous *Lygosoma quoyi* and many other lizards do not make any mention of the formation of this knob-like plug of luteal tissue in the corpus luteum.

**The Luteal tissue.**—A comparison with the previous stage shows (Ph.M. 7) that there is very great increase in the luteal tissue due to the further hypertrophy of the luteal cells. They are large and active looking and their cytoplasm and nuclei resemble in their histological character those of the previous stage. Careful examination reveals that some of the cells have undergone further cytological changes. A slight increase in the size of the cell is noticed and most of the cells have spherical shape. The nuclei are slightly reduced in size but a large majority present a characteristic vesicular shape and are more intensely staining. Large numbers of luteal cells show the regular system of spherical vacuoles which are more numerous than in the previous stage. Boyd (1940) in the account of the luteal cells in the corpus luteum of *Hoplodactylus* mentions the appearance of similar small spherical vacuoles containing lipoid globules. The observations made on the luteal cells of the present form agree with those of the above author. Hett (1924) mentions in his paper on the corpus luteum of *Lacerta agilis* the appearance of vacuoles in the epithelial cells but he does not describe their nature and structure. Extravasated blood corpuscles, some in the process of degeneration, are found in patches among the luteal cells.

**The Theca interna.**—The theca interna is less prominent (Fig. 6). The majority of the nuclei are deeply staining and irregular. There is some decrease in the size of the nuclei of the cells in comparison to the previous stage. These cells form syncytial groups. The septal ingrowths from the theca interna are much more prominent than in earlier stages (Fig. 6 and Ph.M. 7). Extravasated blood corpuscles occur scattered in small patches in the theca interna and along the septal ingrowths as well.

**The Theca externa.**—The theca externa is more reduced in thickness. The line of demarcation between the theca interna and the theca externa continues to be distinct, though not so very clear as in the early stages, while the distinction between the theca compacta and the theca spongiosa is still less definite. Although the septal ingrowths into the luteal tissue take place mainly from the theca interna in the earlier stages, it is clearly seen that strands of connective tissue cells and fibres in close association with the theca interna, are at this period beginning to grow inwards among
the luteal cells. Cell degeneration, evident from the shrunken and collapsed condition of the nuclei of the cells of theca externa are of frequent occurrence at this stage. The large blood-vessels are still confined to the outer region of the theca externa.

Stage V

The corpus luteum as a whole has undergone a further reduction in size and is about 10 mm. in length and the embryo relating to the corpus luteum is 100 mm. in length.

It is a well-developed solid glandular organ densely packed with luteal cells (Ph.M. 8). The central lumen is completely obliterated. The only indication of the initial opening is a shallow groove on the surface. The connective tissue sheath has grown over the projecting plug of luteal cells.

The Luteal tissue.—The luteal cells are more closely packed together than in the previous stage (Ph.M. 9). They appear to be smaller and many of them have assumed a more elongated shape although the nuclei appear unchanged. Hence this reduction in size and the elongated shape of the cells may be due to the mutual compression of the cells, caused by the further shrinkage that the corpus luteum has undergone as a whole. The cells are glandular and active. The small spherical vacuoles in the cytoplasm mentioned in the previous stage have become slightly more numerous. The staining capacity of the nuclei is the same in all the cells and all of them take a deep stain.

The Theca.—There is a considerable reduction in the thickness of the sheath tissue (Ph.M. 9) and the distinction between the theca externa and theca interna comprising the connective tissue wall of the follicle cannot be made out either at this stage or hereafter. The reduction in the thickness of the theca may be due to the degeneration of cells occurring in the theca externa in the previous stage and also on account of a superficial penetration of the connective tissue cells of the theca interna between the luteal cells. The invasion of the thecal cells and fibres takes place as radial ingrowths but they do not extend very far into the luteal tissue. The blood vessels are still confined to the outer region of the theca.

Stage VI

The corpora lutea described in the three following stages were taken from a female in which the embryos had grown to a larger size measuring approximately 120 mm. The corpora lutea are smaller than in the previous stage. All the three were of equal size measuring 9 mm. in length approximately.
Although the embryos in the uterus do not differ in any marked degree in size and development, the corpora lutea represent three distinct stages in the degeneration of the gland. It is therefore highly probable that once the degeneration processes have started there might be considerable variation in the rate or progress of these changes.

The corpus luteum at this stage shows further reduction. Degeneration has commenced in the luteal tissue. The histological character of many of the luteal cells has altered greatly. Luteal cells showing various stages of degeneration, ranging between fairly normal cells with distinct nucleoli and chromatin network, and shrunk cells with densely staining irregular nuclei and highly vacuolated cytoplasm are seen throughout the whole mass of the luteal tissue (Fig. 7). Weekes (1934) observes vacuolization in the cytoplasm of the luteal cells in the oviparous Amphibolurus muricatus and in the viviparous lizard Lygosoma (Hinulia) quoyi. In the latter, towards the latter half of pregnancy the vacuoles become very much enlarged. She infers degeneration from the vacuolated appearance of the cell cytoplasm, the breaking down of the nuclear membrane and the general shrinkage and collapse of all the nuclei. A great majority of the luteal cells have undergone shrinkage and both cell and nuclei are smaller than in the preceding stages. There is a marked increase in the number of intra-cellular vacuoles which are larger, irregular and of varying sizes in contrast to the minute spherical vacuoles of the earlier stages. The cytoplasm of a large majority of cells is completely filled with them. Further evidence of degeneration is seen in the distortion of the nuclei which seems to take place before the loss of the nuclear membrane. Most of them are crenated and shrunken. Clumping of chromatin of these nuclei is evident from the densely staining condition where neither the nucleoli nor chromatin network could be made out. Vacuolar spaces containing pyknotic nuclei and nuclear fragments are not rare.

The connective tissue theca has changed but slightly. It is hardly to be distinguished from the surrounding ovarian stroma. The nuclei of the connective tissue theca cells appear to be smaller than those of the previous stage. The blood vessels, as compared with the early stages, are very much reduced both in number and size, and they are still concentrated in the outer region though a few blood-capillaries are found scattered in the region of the theca interna.

**Stage VII**

The degeneration of the luteal cells once begun continues until it has spread throughout the corpus luteum. The luteal tissue exhibits a spongy
appearance, owing to the presence of large intracellular vacuoles and numerous spaces in between the cells (Ph.M. 10). Varying phases of

**Figs. 6-9.**—Fig. 6. T.S. of the corpus luteum, Stage IV, showing the great increase in the luteal tissue and prominent ingrowths of the theca interna. Fibres from the theca externa are seen to grow along with the theca interna ingrowths. ×80. Fig. 7. T.S. of the corpus luteum, Stage VI, showing the reduction in the thickness of the sheath-tissue and the change in the luteal cells as a result of degeneration. Some have become greatly reduced in size. ×80. Fig. 8. T.S. of the degenerating corpus luteum showing the inter-cellular spaces, the coagulum formed by the degeneration of luteal cells and the reduced theca. ×80. Fig. 9. T.S. of corpus luteum in an advanced stage of degeneration showing the great increase of inter-cellular spaces and the appearance of leucocytes among the degenerating luteal cells. ×80.
regression of luteal tissue can be seen. Many of the luteal cells have undergone further shrinkage and some of them have assumed a more elongated appearance. In the centre of the corpus luteum can be seen some cells with fairly normal structure, containing vesicular nuclei and granular cytoplasm. There is an appreciable increase in the vacuoles in the cytoplasm. Several of them even join together to form one or two big vacuoles which push the nucleus to one side and the whole cytoplasm is seen as a ring round the vacuole.

Further evidence of degeneration can be noted. Darkly staining masses have appeared in the cells which have now lost their cell limits and in which the nuceli have become shrunken and pyknotic. Many of these degenerating cells group themselves together in places. Some of them apparently transform themselves into a structureless coagulum, probably as the result of a further degeneration of these nucleated masses (Fig. 8 and Ph.M. 10). Hill and Gatenby (1926) in *Platypus*, Hett (1924) in *Lacerta agilis*, and Weekes (1934) in *Lygosoma (Hinulia) quoyi* have observed a corresponding coagulum in the luteal tissue and regarded them as products of cell degeneration. Another evidence of degeneration is seen in the presence of vacuolar spaces containing spherules of varying size and faintly staining capacity which have appeared inside the degenerating luteal cells. Similar vacuolar spaces containing colloidal spherules of a finely granular structure in the degenerating corpus luteum are described in *Platypus* by Hill and Gatenby (1926). Vacuolated cells, in which the vacuoles are devoid of formed matter or granular spherules, with irregular shrunken nuclear bodies are very frequent.

The connective tissue sheath is now reduced to such an extent that it is seen as a thin envelope and in certain regions it is only about 2 or 3 cells in thickness (Fig. 8 and Ph.M. 10). There is absolutely no distinction between theca interna and theca externa. At the regions where it is in contact with the ovary it is very indistinct and hardly to be distinguished from the surrounding ovarian stroma. The reduced blood vessels are confined to the periphery.

*Stage VIII*

The corpus luteum has undergone the final regressive changes (Ph.M. 11). The cell cytoplasm and nuclei of the luteal cells are in an advanced stage of degeneration. The degeneration is more marked at the periphery than in the central region. A comparison with the earlier stages shows at a glance the widespread degeneration of the luteal cells and the reduction they have undergone. Consequent on the reduction in size and number of cells there is a considerable increase in the number of intercellular spaces
which have become greatly enlarged (Fig. 9 and Ph.M. 12). The vacuoles in the cytoplasm have become very prominent and larger. In the great majority of cells the cytoplasm is very much reduced and all that is left is cell membrane with a thin pellicle of protoplasm enclosing a large single vacuole with the nucleus situated on one side at the periphery. Simultaneous with the degeneration of the luteal tissue large spaces containing leucocytes appear among the regressing luteal tissue (Fig. 9 and Ph.M. 12). They are probably phagocytic in nature and may be concerned with the removal of the degenerated luteal cells in the same way as described by Sandes (1903) in Dasyurus viverrinus and by O'Donoghue (1916), in Didelphys aurita.

The connective tissue sheath is very thin and reduced which may be another result of degeneration (Fig. 9 and Ph.M. 12). In Enhydrina degeneration seems to set in as early as Stage VI, and is well advanced in Stage VIII.

Summary

1. Enhydrina schistosa, a sea-snake of the Madras Coast, is viviparous with a highly specialised allanto-placenta. Gravid females were obtained from November to December, 1940, and several ruptured follicles in different stages of the formation of the corpus luteum were studied.

2. The structure of the wall of the mature follicle is described. As in mammals the three layers of the wall of the Graffian follicle concerned in the formation of the corpus luteum, after ovulation are (1) the follicular epithelium, (2) theca interna, (3) theca externa. The histological changes taking place inside the ruptured follicles leading up to the formation of a solid gland and its subsequent degeneration are described in detail.

3. The luteal cells are exclusively formed from the small cells of the follicular epithelium.

4. The theca is greatly thickened after ovulation and shows a clear distinction into theca interna and externa and the latter into theca compacta and spongiosa. There is a gradual reduction of the theca in the later stages accompanied by decrease in vascularity.

5. Ingrowths of cells and fibres from the theca among the luteal tissue take place but these are not accompanied by blood vessels. The fibroblastic cells do not penetrate in between the individual luteal cells.

6. In Enhydrina schistosa the active phase of the corpus luteum is comparatively short as degeneration sets in early.
Hydrophis cyanocinctus (Daudin)

*Hydrophis cyanocinctus* is another viviparous sea-snake of the Madras Coast. The material studied was collected from 5 gravid females and one in the post pregnant stage early in 1940. The breeding season probably centres toward March, April and May. The ovary of this species resembles that of *Enhydrina schistosa* in all essential respects, except in the comparatively smaller size of the ova. Two to nine ova attain full size in each ovary simultaneously, three being the usual number of embryos found in each uterus corresponding to the number of corpora lutea in the ovary.

**The Structure of the Wall of the Unruptured Follicle**

As in *Enhydrina schistosa*, the egg is surrounded by the zona radiata and the vitelline membrane. The wall of the unruptured follicle of snakes has been described in *E. schistosa* and the condition in *H. cyanocinctus* is essentially similar in all the main histological and cytological details. It differs from the former in a few minor details which are given below.

*The Follicular epithelium.*—The follicular epithelium is a well-defined layer varying from 3 to 4 cells in thickness. It possesses two kinds of cells—large specialised cells, wedged in among which are small cells as in the case of *E. schistosa*. The large follicle cells have prominent vesicular nuclei with a large nucleolus and a network of deeply staining large chromatin granules. The cytoplasm is massive and deeply staining. The majority of large cells are pear-shaped (Fig. 1, Ph.M. 1) and are arranged with their long axes radial to the surface of the egg, their narrow pointed ends being directed towards the surface. The smaller cells possess fairly spherical nuclei, with a nucleolar reticulum. A large number of these small undifferentiated cells lie close to the membrana propria.

The membrana propria considered by most authors as a modified layer of theca folliculi is well marked and distinct from the rest of the theca. It consists of a thin layer of elongated cells with elongated nuclei densely filled with fine chromatin granules (Fig. 1 and Ph.M. 1).

*The Theca folliculi.*—The theca folliculi is on the whole not so thick as in *E. schistosa*. In *E. schistosa* the theca folliculi is specialised into two layers, an outer fibrous layer, the theca externa and an inner layer the theca interna containing a large number of connective tissue cells. But in *H. cyanocinctus* this differentiation of the theca folliculi into theca externa and theca interna is extremely slight. The rudimentary theca interna, however, is represented by a narrow layer of two or three cells thickness, containing
connective tissue cells with oval or elongated nuclei and fibres running in between them. The cells and fibres are arranged parallel to the follicular epithelium. The theca externa layer surrounding the inner theca is arranged in several layers and consists of connective tissue fibres and occasional fibroblastic cells with elongated nuclei, both of which are disposed parallel to the inner layers. In association with this layer there are a few minute blood vessels.

Corpus luteum

Stage I

The youngest corpus luteum obtained corresponds to an embryo at an early stage of development. It is oval in shape and measures only about 5 mm. in length.

There is a lumen in the centre of the corpus luteum, 'the antrum folliculi' lined by the follicular epithelium, and filled with an honey-combed mass of deeply staining coagulum, intermingled with a few cells. These cells with large spherical nuclei are identical with those forming the follicular epithelium. Evidently they are detached from the follicular epithelium by the rupture and escape of the ovum.

The Luteal tissue (Fig. 2).—As a result of ovulation the follicle as a whole has undergone contraction and the follicular epithelium is thrown into folds. As described in Enhydrina the follicular epithelium, in contrast to the unruptured condition, consists of only one kind of cells—the small cells, the large cells having completely disappeared. There is considerable hypertrophy of the follicular epithelium. It is approximately twice the thickness of that of the unruptured follicle and consists of large moderately staining cells of variable shapes, spherical or polygonal with distinct cell boundaries. During the process of transformation of the follicular epithelial cells into luteal cells changes take place that concern both the nucleus and the cytoplasm. The nucleus which hitherto remained small, gradually enlarges and assumes a characteristic vesicular shape, and contains one or two nucleoli. As the luteal condition is assumed nucleoli become more and more a prominent feature of the nuclei. A slight variation in the staining capacity of these cells is noticed. Some of the cells take a light stain while others are intensely stained. The cytoplasm stains moderately and is finely granular, the granules being diffusely scattered throughout the cytoplasm and they show up fairly with iron alum haematoxylin.

The Theca (Fig. 2).—Coincident with these processes in the follicular epithelium, certain histological changes take place in the theca folliculi. The membrana propria is no longer seen as a distinct layer. After the rupture
of the follicle, the theca is much more thickened which may be due to shrinkage of the follicle. In comparison with the remarkable thickening and differentiation of the theca folliculi into a distinct theca interna rich in connective tissue cells and a fibrous theca externa in *E. schistosa*, the distinction here is comparatively slight.

*The Theca interna.*—The theca interna is seen as a narrow layer close round the follicular epithelium and follows the wavy contour of the epithelial layer. This layer consists of connective tissue cells and fibres running in between them (Fig. 2). The theca interna cells are totally unlike the luteal cells of the follicular epithelium and can be readily distinguished from them. The cells of the theca interna at no time approximate to the luteal cells in size. But their average size is larger than in the unruptured condition. Their bounding cell membranes are usually indistinct, their nuclei vary from oval to elongate and they stain rather deeply and show a nucleolar body and a reticulum of minute chromatin granules. The cytoplasm stains moderately and is frequently vacuolated. This fibrocellular layer varies in thickness and the cells and fibres run parallel with the surface of the follicular epithelium. At this stage there is no sign of any inward growth of the connective tissue from the theca. It is during this period that the theca interna reaches its maximum development. As the corpus luteum advances in development the differentiation of the theca folliculi into theca externa and interna becomes practically indistinguishable and the latter is only represented by an indistinct layer of small oval nucleated cells and fibres, closely resembling the theca externa lying close to the follicular epithelium.

*The Theca externa* (Fig. 2).—The theca externa surrounding the follicle has increased in thickness after the rupture and is many times thicker than the theca interna. In *E. schistosa* the theca externa is differentiated into two layers, an inner compact layer called the theca compacta and an outer irregular one containing the large blood-vessels, the theca spongiosa. In *H. cyanocinctus* this differentiation of the theca externa into theca compacta and theca spongiosa is extremely slight. The outer region of the theca carries a few small blood vessels. In the connective tissue substance of the theca externa nuclear degeneration could be clearly noticed. Some of the fibroblastic cells of the theca externa can be seen surrounding the walls of the blood vessels. Vascularisation is very slight in earlier stages but gradually increases in later stages accompanied by a greater development of the connective tissue sheath.
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Figs. 1-6.—Fig. 1. T.S. of the unruptured follicle showing the follicular wall. ×120. Fig. 2. T.S. of the corpus luteum, Stage I, showing the three layers. ×80. Fig. 3. T.S. of the corpus luteum, Stage II, showing the increase in the luteal tissue and reduction in the theca and the ingrowth of connective tissue. ×80. Fig. 4. T.S. of the corpus luteum, Stage III, showing the great increase in the luteal tissue and the ingrowth of connective tissue septa and the fibres cutting the luteal cells into 'nests'. ×80. Fig. 5. T.S. of the corpus luteum, Stage IV, showing the prominent ingrowth of the connective tissue septa and the invasion of blood vessels along with it. ×80. Fig. 6. T.S. of the corpus luteum, Stage V, showing the extensive development of the septum which carries the blood vessels and the penetration of fibroblastic cells among the individual luteal cells. ×80.
Stage II

The corpus luteum is more or less spherical and is about 6 mm. \(\times\) 5 mm. in diameter. The embryo relating to the corpus luteum described in this stage is about 122 mm. in length.

This stage shows a further advance. The whole gland has slightly increased in size. There is a large oval lumen in contrast to the compressed elongated lumen observed in *E. schistosa* (Ph.M. 2). Now there is no trace of the deeply staining coagulum in the lumen. The opening of ruptured spot is occupied by a mass of luteal cells which is more or less exposed and partially devoid of connective tissue covering. The rapid enlargement of the luteal cells in a limited space must have caused the protrusion of the cells into the opening left by the escape of the egg. A corresponding protrusion of the luteal tissue has been described in *E. schistosa* but the luteal plug is smaller in this case because of the enlargement, the follicle undergoes as a whole. In *H. cyanocinctus* the ruptured opening is almost immediately closed by a plug of luteal cells and the coming together of the connective tissue wall.

The Luteal tissue.—The luteal tissue is more massive and the thickness of the epithelial wall of the corpus luteum as shown in a transverse section has increased appreciably (Fig. 3 and Ph.M. 2). It is almost thrice the thickness of that of the previous stage, the increase being due mainly to the simple hypertrophy of the individual cells composing it. They vary in shape from spherical to polygonal and have distinct cell limits as in the previous case. The cytoplasm generally appears in reticulate form and stains moderately. The nucleus has become slightly larger and typically vesicular and contains a large karyosome, usually two nucleolar bodies and a distinct chromatin reticulum. The variation in the staining capacity of the nuclei is still more prominent.

The Theca (Fig. 3 and Ph.M. 2).—The theca has undergone a marked stretching and consequent reduction in thickness due to the enlargement of the luteal cells. The line of demarcation between the theca interna and theca externa is less distinct but there is slight increase in the number of blood vessels, which are apparently confined to the theca externa. From the theca interna connective tissue fibres and cells are in the process of invading the luteal tissue (Fig. 3).

Stage III

The corpus luteum has an oval shape and measures 10 mm. in length. The embryo in the uterus corresponding to the corpus luteum studied is 142 mm. in length.
Photomicrograph 3 shows that the central lumen is further reduced by the growth of the luteal cells towards the centre. The connective tissue has covered over the plug of luteal cells.

The Luteal tissue (Fig. 4 and Ph.M. 3)—There is an increase in thickness of the luteal tissue due to mere hypertrophy of the cells as no mitosis has been observed. This leads to the gradual filling up of the central lumen. The luteal cells have attained a larger size with relatively large vesicular or oval nuclei. The nuclei, rich in chromatin granules, stain more deeply than in the previous stage but the variation in the staining capacity is less. The cytoplasm of a few cells shows a regular system of minute spherical vacuoles round the nuclei.

The Theca.—The distinction between the theca interna and externa is still less definite. At the periphery of the corpus luteum the luteal tissue is cut into ‘nests’ of luteal cells by the ingrowth of connective tissue fibres from the theca interna (Fig. 4 and Ph.M. 3). Although the theca interna is very much reduced and hardly distinguishable from the theca externa, it can be seen pushing inwards among the luteal cells as radial ingrowths. This reduction of the theca interna may be due to the ingrowths of connective tissue cells and fibres from this layer which have become much pronounced in this stage. A few small blood capillaries are found in this layer.

The most remarkable characteristic of this stage is the increase in number of blood vessels of considerable size in the theca externa (Ph.M. 3). They are found to be far more abundant than in any of the previous stages. The large blood vessels are concentrated at the periphery of the theca externa where they are surrounded by strands of fibroblastic cells of the theca externa. Although the radial ingrowths into the luteal tissue are chiefly from the reduced theca interna, strands of fibroblastic cells from the theca externa in close association with those of the internal layer are at this stage beginning to grow inwards.

Stage IV

The corpus luteum is about the same size as in the previous stage but the embryo has reached a length of 190 mm.

The Luteal tissue.—A transverse section (Ph.M. 4) of this stage shows a still greater advance. The luteal cells are slightly larger than in the previous stage but the cytological character of most of the cells remains practically unchanged. There is a slight increase in number of the minute spherical vacuoles in the cytoplasm of some of the cells. The luteal cells
have grown enormously and almost obliterate the lumen which can be observed only as a narrow slit in a few sections passing through the centre of the corpus luteum.

The Theca.—The theca interna has undergone further attenuation and is hardly distinguishable from the theca externa. There is a corresponding thinning of the sheath tissue. The connective tissue fibres have grown inwards among the luteal cells from all over the inner surface of the theca interna and as a result, the number of ‘nests’ of luteal cells at the periphery have become very numerous (Fig. 5 and Ph.M. 4).

The corpus luteum exhibits a radially divided appearance which is emphasised by the large strands of fibroblastic radial septal ingrowths from the theca (Fig. 5 and Ph.M. 4). An important feature of the corpus luteum at this stage as illustrated in Ph.M. 4 and Fig. 5 is that the large blood vessels in association with the fibroblastic septal ingrowths of the theca externa, which were hitherto confined to the periphery of the theca externa have now grown into the luteal tissue. The connective tissue fibres alone penetrate from the theca interna between the luteal cells, as mentioned above, and form a network among them (Fig. 5 and Ph.M. 4).

Stage V

In the next stage of development the corpus luteum has increased in size and is approximately 12 mm. in length while the embryo relating to this stage is 195 mm.

The corpus luteum has become solid throughout and has attained the height of its histological differentiation (Ph.M. 5).

The Luteal tissue.—The luteal cells are glandular and active and are larger than those of the preceding stages (Ph.M. 6). The cell boundaries are distinct. The spherical cytoplasmic vacuoles have greatly increased in number and in a few cells the whole cytoplasm is filled with them. The nuclei are large and prominent and contain a large irregular karyosome and one or two smaller nucleoli with all of which a network of deeply staining chromatin granules is connected. When the nucleus has reached its maximum growth it presents a striking vesicular appearance (Ph.M. 6). Some of the nuclei take a deeper stain than others but this variation in the staining capacity is not so marked as in some of the earlier stages. No nuclear degeneration has been noticed in any of these cells. There is a greater increase of luteal tissue as compared with the earlier stages. Since there is no evidence of cell division either mitotically or amitotically we come to the conclusion that the increase has been mainly brought about by simple
hypertrophy of the luteal cells. If mitotic division occurs at all it must be
limited for a short time to the early stages, just after ovulation.

_The Theca._—Even though the hypertrophy of the luteal cells is the
main factor in bringing about the solidification of the corpus luteum in the
later stages, the ingrowth of connective tissue from the theca together
with the blood vessels also facilitates the process.

The septa of fibroblastic cells from the theca have penetrated through-
out the luteal tissue carrying blood vessels (Fig. 6 and Ph.M. 5). Conse-
quent on the increase of connective tissue ingrowth among the luteal cells,
the outer sheath has undergone reduction in thickness and is seen as a thin
envelope, 2 to 3 cells thick in certain regions. The connective tissue
fibroblastic cells and fibres grow in all directions so that the luteal cells
become surrounded by an anastomosis of these elements (Fig. 6 and
Ph.M. 6). Thus the connective tissue elements of the theca contribute to
the formation of the supporting framework for the luteal cells and the
the blood vessels.

**Stage VI**

The corpus luteum is kidney-shaped and is considerably smaller than
in the preceding stage. It measured 6 mm. Since no embryo was obtained
it appears that the uterus is in the post-pregnant stage. Though very
small in size the corpus luteum was readily distinguishable from the white
ova by its yellow colour. A careful study reveals signs of degeneration in
some of the luteal cells. Those towards the centre have undergone
shrinkage and are smaller than the similarly situated cells described in
Stage V. Cells with deeply staining crenate nuclei are occasionally seen,
their presence indicating commencement of degeneration in luteal cells.
There is an appreciable increase in the size of the vacuoles in the cytoplasm
which have now become irregular. Another evidence of degeneration is
seen in the appearance of inter-cellular spaces in the middle of the luteal
tissue. Faintly staining masses of coagula could be seen here and there.
These are all indications that the degeneration processes are in full
operation.

The connective tissue theca does not appear to have changed in any
appreciable manner. But in the meanwhile the inter-epithelial connective
tissue has become more finely distributed.

**Summary**

1. The formation of the corpus luteum in _Hydrophis cyanocinctus_,
another viviparous snake with allanto-placenta, is fundamentally similar to
that of *Enhydrina schistosa* in all essential features. The luteal cells are derived by hypertrophy from the small cells of the follicular epithelium.

2. There is a very slight distinction between the theca interna and theca externa. The fibroblastic cells and the fibres of the theca show greater development with a simultaneous increase in the later stages of the number of blood vessels accompanied by extensive ingrowths of connective tissue septa carrying blood vessels into luteal tissue together with penetration of fibroblastic cells in between the luteal cells.

3. The corpus luteum in the present form has a long intra-ovarian existence and signs of degeneration of luteal cells are noticed only after the birth of the young.

**Discussion**

Despite the difference in their zoological position the reptiles and mammals show considerable agreement in the development and structure of the corpus luteum. In reptiles as in the mammals, the three layers of the wall of the ruptured follicle, the follicular epithelium, theca interna and theca externa are concerned in its formation. The corpus luteum of the viviparous sea-snakes offers marked advantages for the observation of the changes undergone by the different layers. The corpus luteum in the fully developed condition is a solid glandular organ formed by the hypertrophy of the small cells of the follicular epithelium reinforced with the connective tissue from the theca.

The histological structure and appearance of the corpus luteum in *Enhydrina schistosa* and *Hydrophis cyanocinctus* are fundamentally similar. The corpus luteum in *Enhydrina schistosa* is the largest yet described among the reptiles. In *H. cyanocinctus* it is smaller in the early stages but gradually enlarges though in no stage does it exceed the size of the corpus luteum in *E. schistosa*.

**Luteal cells.**—The luteal cells are derived exclusively from the follicular epithelium in both the forms, in striking contrast to the condition in *Rhinobatus granulatus* (Samuel, 1943). Mitotic divisions have been recorded in the follicular epithelial cells in the early stages of the formation of the corpus luteum in certain marsupials (O'Donoghue, 1914 and 1916), in sheep (Marshall, 1904) and in the lizard *Amphibohurus muricatus* (Weekes, 1934). Hett (1924) who worked on the corpus luteum of *Lacerta agilis* and Boyd (1940) who studied the gland in *Hoplodactylus maculatus* have however, failed to observe mitotic divisions among the luteal cells, in the forms studied and it is obvious, that if there is any mitosis at all it cannot be very
extensive. The major portion of the increase in the luteal tissue must therefore be brought about by simple hypertrophy of the cells.

Our knowledge of the cytological structure and secretory activity of the luteal cells is very limited. There seem to be two phases in the deposition of fat in the corpus luteum of snakes, viz., a lipoidal secretion and a later formation of fat as in the higher mammals. An important feature of the Eutherian luteal cells is the presence of secretory globules. In material fixed in Bouin and corrosive acetic these are present after the usual histological procedure as chains or groups of regular, empty vacuoles. It was not possible for me to study the secretory history of the luteal cells due to scarcity of material. However, the presence of small spherical vacuoles of equal size and similar shape suggests that globules secreted in the fully mature, active corpus luteum are of a lipoid nature. They seem to represent the lipoid secretions of the higher vertebrates. These lipoid globules are readily removed in the non-osmic fixatives used and in the subsequent procedure, and the position of these globules is marked by a regular system of minute spherical vacuoles.

The degree of development of these spherical vacuoles in H. cyanocinctus is greater than that in E. schistosa. This seems to be correlated with the longer functional activity of the corpus luteum in H. cyanocinctus in contrast to the lesser development and apparently shorter functional life of the luteal cells in E. schistosa. These vacuoles are extremely small and inconspicuous in comparison with similar vacuoles observed in Rhinobatus. In view of the constant occurrence of such globules in the luteal cells of mammals, their presence in the snakes is of interest.

The later appearance of large irregular vacuoles of various sizes and shapes, lacking the uniformity present in the early stages, may be due to the deposition of fat as a sign of fatty degeneration of the cells. This is further supported by the fact that the appearance of these large irregular vacuoles is accompanied by degenerative changes in the nuclei.

Boyd (1940) describes in the luteal cells of Hoplodactylus maculatus, the presence of minute vacuoles about the nucleus, occupied by a lipoid substance which blackens with osmium tetroxide. In later stages of the corpus luteum, Boyd further states that “the luteal tissue is very vacuolated, both the size and number of the vacuoles having increased, giving the tissue the appearance of a network”. Observations made on the luteal cells of the present forms agree with those of the above author. Hett (1924) records the appearance of vacuoles in the luteal cells of Lacerta agilis, both in early and late stages, but he does not express any opinion regarding the
nature of the secretory products of the luteal cells. Weekes (1934) mentions the appearance of large vacuoles in the cytoplasm of luteal cells in the later stages both in Amphibohurus muricatus and Lygosoma (Hinulia) quoyi. According to this author, these vacuoles become very pronounced at the time of the birth of the young. In her opinion the appearance of large vacuoles in the cytoplasm together with the breaking down of the nuclear membrane and general shrinkage and collapse of the nuclei is a sign of regression of the tissue. Weekes, however, does not mention the presence of a regular system of minute spherical vacuoles. This may be attributed to the minute size of these vacuoles.

The Theca.—The structure and subsequent behaviour of the theca folliculi vary greatly in the two species studied. In E. schistosa immediately after ovulation, the theca is greatly thickened and differentiated into theca interna and theca externa. The theca externa further shows distinction into theca compacta and theca spongiosa. In this respect E. schistosa approaches the condition described in mammals and agrees with lizards such as Amphibohurus muricatus, Lygosoma (Hinulia) quoyi (Weekes, 1934), and Lacerta agilis (Hett, 1924). This distinction between the theca interna and theca externa is fairly well marked and constant up to a late stage.

Even the little distinction that exists between the theca externa and theca interna in the early stages in H. cyanocinctus is lost in the later stages. Another point of difference is that the connective tissue fibres and fibroblastic cells are very compactly arranged in the theca in H. cyanocinctus while they are loosely scattered in E. schistosa. These variations seem to be pretty common. Boyd (1940) for instance has called attention to the fact that distinction between the theca interna and theca externa does not occur in Hoplodactylus. O'Donoghue (1914) observes great variation in the degree of development of the theca interna in Marsupials. In Phascolomys, the theca interna is well developed, while it is extremely rudimentary in Dasyurus.

Variations occur in regard to the ingrowth of the theca into the luteal tissue, in the two species studied. In E. schistosa in the early stages, connective tissue septal ingrowths of cells and fibres unassociated with blood vessels takes place from the theca interna into the luteal tissue and later strands of connective tissue cells and fibres from the theca externa also join in this invasion. The connective tissue fibres and cells in E. schistosa do not form an anastomosis of fusiform cells surrounding the luteal cells as in H. cyanocinctus. In H. cyanocinctus there is an extensive ingrowth of connective tissue septa carrying large blood vessels. The fibres and fibroblastic cells invade among the luteal cells and nearly all the cells become surrounded by a network of fibroblastic cells and fibres.
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Weekes (1934) distinguishes three kinds of behaviour of the theca in the lizards. In L. quoyi, L. quadridigitatum and E. whitei there is no invasion of theca interna fibroblastic cells in amongst the individual luteal cells. In L. Weekeae and L. entrecasteauxi, there is ingrowth of strands of fibroblasts in association with blood vessels, but they do not penetrate between the individual luteal cells and thirdly as seen in L. vivipara, the spetal growth carrying the blood vessels and a penetration of fibroblastic cells among the luteal cells takes place.

Boyd states that Lacerta vivipara and Hoplodactylus (Boyd, 1940) "are the only ones so far in reptiles where fibroblasts penetrate between the individual lutein cells as well as form septa", but that "In Lacerta vivipara, however, blood-vessels grow in with the connective tissue". It is noteworthy that in Hydrophis cyanocinctus we find both the penetration of the fibroblastic cells among the luteal cells and the ingrowth of the blood vessels in association with fibroblastic septa. Hence the similarity between the L. vivipara and H. cyanocinctus is more complete. In regard to the ingrowth of connective tissue theca, Enhydrina schistosa shows similarity to Lygosoma quoyi, in which there is only a superficial ingrowth of fibroblastic cells among the luteal cells, and no blood vessels are found among them.

Another feature of interest is the difference in the time of vascularisation of the corpus luteum in the two forms studied. In E. schistosa, soon after ovulation, there is a great development of blood vessels in the greatly thickened theca externa which becomes gradually reduced in later stages; on the other hand in H. cyanocinctus the theca is poorly vascularised in the early stages but vascularisation increases later accompanied by a greater development of the theca. It has been suggested by Deanesly (1930 b) for the mammals that this variation in the development of the theca and blood vessels can be attributed to the difference in size of the ripe follicle.

Although there is fundamental similarity in the structure of the corpus luteum in both the forms, the duration of functional activity of the gland in relation to the development of the embryo in the uterus shows considerable difference in the two forms studied. In E. schistosa the corpus luteum attains its full growth when the embryo is about 44 mm. and becomes solid by the time the embryo attains a length of 100 mm. Regression sets in shortly after and is well advanced when the embryo is about 120 mm. Degenerative changes once begun, advance rapidly and the final stages of degeneration are in evidence even when embryos are of the same length. In H. cyanocinctus the corpus luteum is at its height of histological development when the embryo is 195 mm. and evident signs of degeneration are observed only after the birth of the young.
In the viviparous lizards, Weekes (1934) states that the corpus luteum has an intra-ovarian existence throughout the gestation period and disappears only after the birth of the young. According to Boyd (1940), in Hoplodactylus the corpus luteum remains in the ovary even after the birth of the young though degeneration has set in. With regard to the long intrauterine existence of the corpus luteum H. cyanocinctus resembles the viviparous lizards while E. schistosa shows resemblance to the monotremes where 'regression appears to set in remarkably early' (Hill and Gatenby, 1926). Apparently the functional activity of the corpus luteum is most important only in the early stages of development of the embryo as otherwise it is difficult to explain its later behaviour in the various animals studied.

A review of our present knowledge of the corpus luteum in reptiles in general, will be given in a paper on the corpus luteum of another viviparous snake, Cerberus rhyncops, under preparation.

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N.B.—* Not referred to in the original.

EXPLANATION OF PHOTOMICROGRAPHS

PART I

PLATE IV

Photomicrograph 1. T.S. of a fairly mature egg showing the two kinds of cells of the follicular epithelium.

2. T.S. of the wall of the fully matured follicle showing the degeneration of large cells and the thickened theca.

3. T.S. of the corpus luteum, Stage I, showing the rupture.

4. T.S. of the corpus luteum, Stage I, showing the three layers of the wall follicular epithelium, theca interna and theca externa with the large blood vessels.
Photo-
micrograph 5. T.S. of the corpus luteum, Stage III, showing the ruptured opening.
6. T.S. of the corpus luteum, Stage III, showing the wall and ingrowth of theca
   interna elements into the luteal tissue.

PLATE V

Photo-
micrograph 7. T.S. of the corpus luteum, Stage IV, showing the reduced central lumen and
   the increase in the luteal tissue.
8. T.S. of the solid corpus luteum, Stage V.
9. T.S. of the corpus luteum, Stage V, showing a portion to illustrate the closely
   packed luteal cells.
10. T.S. of the degenerating corpus luteum, Stage VII, showing the coagulum
    in the luteal tissue, inter-cellular spaces in the luteal tissue, and the
    reduced theca.
11. T.S. of the degenerating corpus luteum, Stage VIII, presenting a highly
    degenerate stage of luteal cells.
12. T.S. of the corpus luteum, Stage VIII, a portion magnified to show the large
    number of intercellular spaces and the appearance of leucocytes among
    the degenerating luteal cells.

PART II

PLATE VI

Photo-
micrograph 1. T.S. of the unruptured follicle showing the pear-shaped large cells, and the
   small cells of the follicular epithelium.
2. T.S. of the corpus luteum, Stage II.
3. T.S. of the corpus luteum, Stage III, showing the ingrowth of connective
tissue septa and the increase in luteal tissue.
4. T.S. of the corpus luteum, Stage IV, showing the prominent septa and the
   invasion of blood vessels along with them and the ‘nests’ of luteal cells.
5. T.S. of the corpus luteum, Stage V.
6. The luteal cells of Stage V magnified to show the large size of the cells
   and the network of fibroblastic cells surrounding the luteal cells.

KEY TO LETTERING

b.v. .. Blood vessels.  le. .. Leucocytes.
cm.t.i.g. .. Connective tissue ingrowth.  m.p. .. Membrana propria.
cm.t.se.i.g. .. Connective tissue septal in-
growth.  ne.l.c. .. Nests of luteal cell.
co.l.t. .. Coagulum in luteal tissue.  ov.w. .. Ovarian wall.
pl.l.t. .. Plug of luteal tissue.  ro. .. Ruptured opening.
d.l.c. .. Degenerating luteal cell.  s.c. .. Small cell of the follicular
   epithelium.
   d.l.t. .. Degenerating luteal tissue.  th.c. .. Theca.
   d.l.a.c. .. Degenerating large cell of the
   follicular epithelium.  th.ex. .. Theca externa.
   f.e. .. Follicular epithelium.  th.fl. .. Theca folliculi.
   b.c. .. Fibroblastic cells.  th.i. .. Theca interna.
   ind.l. .. Intercellular species in the
   luteal tissue.  th.sp. .. Theca spongiosa.
   l.e. .. Luteal cell.  z.r. .. Zona radiata.
   l.t. .. Luteal tissue.
   l.a.c. .. Larger cell of the follicular
   epithelium.

N.B.—All figures have been reduced to half the dimensions given.