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TITLE CYTOLOGICAL STUDIES ON THE OÖGENESIS OF CERTAIN INDIAN AND AMERICAN SNAKES

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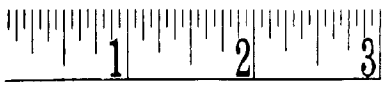

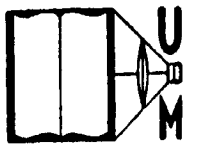

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BRAHMA SWARUP KAUSHIVA

1949

CYTOLOGICAL STUDIES ON THE OÖGENESIS OF CERTAIN INDIAN AND AMERICAN SNAKES

by

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A dissertation submitted in partial fulfillment
of the requirements for the Degree of
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INTRODUCTION

Although literature dealing with various changes that occur in the cytosome during the developmental history of an egg is extensive and detailed, little consideration has been given to this part of the cell as compared to the nucleus. Interest in chromatin material and its history was naturally stimulated, in part, by the discovery that in it resides the chief hereditary material, and, in part, by the fact that it affords an organization easily accessible to microscopical study. Recent biochemical and cytological evidence indicates that the nucleus, besides controlling heredity, plays an important role as a source, direct or indirect, of some cytoplasmic inclusions which have the capacity to perpetuate themselves in the cytoplasm and materially influence the growth processes of the oöcyte. Cytoplasmic structures which have been extensively studied include: Golgi apparatus, mitochondria, centrosome, "yolk nucleus," and other organoids and inclusions. The part that any of these may play during oögenesis, when the oöcyte progresses from a comparatively low metabolic state to a higher level of physiological activity, has not yet been fully determined.

There are several divergent views as to the role played by the cytoplasmic structures in developing eggs. Most investigators (Gatenby, Ludford, Gresson, Harvey, Brambell, Bowen) believe that the growing oöcyte possesses a lipoidal

structure identical with Golgi's "Apparato reticolare interno." On the other hand, some French cytologists (Parat, Hosslet, Voinov) homologize the Golgi apparatus in the oöcyte with the chondriome or the acidic vacuome. They recognize only two living cytoplasmic inclusions in animal cells: the chondriome and the acidic vacuome. They further suggest that the structure identified as the Golgi apparatus in fixed cells is either a modified system of neutral-red vacuoles (vacuome) or specially modified mitochondria called "Lepidosomes" or "Chrondriome actif," the former representing the aqueous phase and the latter the lipoidal phase of a vacuolar system,

Another school of thought takes an extreme view and totally denies the existence of a specific Golgi substance (Walker and Allen, 1927; Tennent, Gardiner and Smith, 1931). According to these workers the Golgi bodies are not independent cell components but are mere artifacts resulting from the action of fixatives on the cephalin and lecithin portions of the cells. In the light of more recent knowledge about these structures, little attention is given to this view at present but it does caution us against hasty conclusions in dealing with them. Such scepticism has been helpful in stimulating the development of new techniques for more convincing demonstration of the actual existence of a Golgi apparatus.

The structure of the Golgi apparatus has been variously interpreted. Some regard it as made up of dictyosomal bodies of crescent-shaped appearance (Harvey, 1925); others speak of it as a vesicular structure (Nath, 1929); still others consider

it a formless fluid rather than an individualized organelle. The main difficulties in deciding upon its morphology have been the different techniques employed by the various workers and the fact that it is not easily visible in the living cell. Apparently the kind and extent of fixation affect markedly the visible form of the Golgi apparatus.

The present work has been extended to include the problem of yolk formation. Some workers ascribe formation of yolk to one or more cytoplasmic inclusions in the oöcyte (Gatenby and Woodger, 1920; Ludford, 1921; Brambell, 1924). Others think that yolk-synthesis takes place in the ground cytoplasm without the intervention of these inclusions (Hibbard, 1928; Harvey, 1929). I have reached the conclusion with regard to the Golgi apparatus that it is responsible for the formation of fatty yolk by direct transformation.

Another structure which apparently plays an important part in the growth of the oöcyte is the "true nucleolus" or plasmosome. However, its active role in the oöcyte is not properly understood at the present time. Some workers have regarded it as a mere ergastic body representing a storehouse of the waste products in the nucleus (Häcker, 1895; Meyer, 1917). Others have described plasmosomal derivatives as passing into the cytoplasm either as discrete bodies or in solution to take part in the formation of proteid yolk. In my work I have demonstrated that plasmosomes migrate from the nucleus to the cytoplasm during growth of the oöcyte.

In the light of our modern concept of heterochromatin-nucleolar-ribonucleic acid relationship developed by Caspersson and Schultz (1940), Brachet (1940), Painter and Taylor (1942), and Painter (1945), it is suggested that these extruded bodies provide the cytological mechanism by which the ribonucleic acid is supplied to the egg cytoplasm.

Some investigators have described the emission of granular material of a true chromatin nature from the nucleus to the cytoplasm (Schaxel, 1911). This view has been held by others to be erroneous (Gatenby and Woodger, 1920). They have doubted the contention that the emitted granules are of a chromatinic nature or that they are even derived from the nucleus. However, Dendy (1915) showed in the eggs of Grantia compressa that solid clumps of chromatin are extruded from the nucleus during oögenesis. Also Gatenby (1920) described similar emission of chromatin material in the oöcyte of Apanteles glomeratus to form secondary nuclei. I have demonstrated the presence of chromatin material in the oöplasm from two sources in the form of "false nucleoli" or karyosomes from the egg nucleus, and as entire nuclei from the follicular epithelium. However, the function of this extruded chromatin material in the metabolism of the egg has not been determined.

The egg follicle thus far has not received sufficient attention in the literature. Little is known about its origin, development and function. In the present study, the formation of the follicle has been traced from the earliest

stages when the oögonium is seemingly devoid of any specialized enveloping membrane.

It is believed that the phenomena connected with the growth of oöcytes are somewhat similar in all vertebrates. In the present investigation, which bears on the extensive changes that take place in the oöcyte during its growth, snakes were selected for study, partly because they could be obtained readily and because cytologically they are perhaps the least explored of the vertebrates. The primary object of this study is to emphasize the combined roles of the nuclear and cytoplasmic bodies of the oöcyte and the follicular epithelium in the growth of the ophidian egg.

Although, this investigation provides a cytological basis for our understanding of certain aspects of biosynthesis, it is conceded that for a study of the entire problem of cellular function we must depend on further development of cytochemical methods such as have recently been employed by Caspersson and Schultz (1940), Mirsky (1943), and others.

ACKNOWLEDGEMENTS

This study was conducted in two stages. A portion of the work was completed in India. The remaining work was done at the University of Michigan. I have received the greatest amount of help from Professor Peter Okkelberg, who directed my research. It is a pleasure to record my sincere thanks to him for his constant help, valuable suggestions, and stimulating criticism. Likewise, I am grateful to Dr. L. P. Mathur, Professor of Zoology, St. John's College, Agra, India, for helping me in the initial stages of this investigation. I am indebted to Dr. B. L. Baker for his kind attention and criticism and to Dr. Norman E. Hartweg for the willing help given on herpetological problems encountered during the progress of this research. Dr. Karl F. Lagler and Dr. Norman E. Kemp have read the manuscript and made several practical suggestions.

HISTORICAL RÉSUMÉ

A general survey of literature on reptiles reveals that there is a paucity of cytological information on reptilian oögenesis, particularly on snakes. Certain aspects of chelonian and lacertilian oögenesis have been studied in some detail but no work dealing with the entire oögenetic phase in snakes has been attempted. Studies to date are incidental to other problems and of a preliminary nature. Very few observations have been made on the anatomical features of the ophidian ovary. Still less attention has been given to its histological structure and seasonal variations during its developmental stages. Besides the work of Cieslak (Unpublished, 1938), I have not found a single paper dealing with this phase of the female reproductive organs in snakes. Volsöe (1944) has written an excellent paper on the structure and seasonal variations of the male reproductive organs in Vipera berus (L.). Similar studies on the female reproductive organs should yield important results and will serve as an introduction to some of the problems in early embryology.

Among the older works dealing especially with the eggs of reptiles mention may be made of that of Rathke on vipers (1839) and, later, on turtles (1848); Lereboullet on lizards (1848); Leydig on fishes and reptiles (1853); and Agassiz on turtles (1857). These workers primarily studied the egg from the point of view of yolk, germinal vesicle and egg membranes.

In one of the earliest papers on the subject, Waldeyer (1870) showed that the follicular epithelium in Lacerta agilis contained two kinds of cells: some small, resembling ordinary follicular cells, and others larger. He described pseudopodia-like processes of the larger cells which penetrate into the yolk through the zona radiata thus serving as channels for the passage of the nutritive material from the follicular cells into the oöplasm. He also described the formation of follicular epithelium from a single layer of cells and noticed a thinning in the zona radiata in older follicles.

Somewhat later, Eimer (1872) found that, during its long period of growth, the ovarian egg has five kinds of membranes: (1) the internal epithelium, "Binnenepithel," lying directly next to the yolk; (2) the vitelline membrane, "Dotterhaut," situated on the outside of the latter; (3) the zona pellucida formed of tiny rods; (4) a kind of chorion; and finally, (5) the follicular epithelium. In the follicular epithelium he distinguished three kinds of cells and expressed the opinion that the striated appearance of the zona pellucida is due to the prolongations from the cells of the follicular epithelium. Eimer's views regarding the internal epithelium were refuted by Ludwig (1874) and Braun (1877). Braun (1877), however, considered the follicular cells of lizards as unicellular glands. He also traced the earlier embryological history of the oöcyte from the primordial germ cell stage.

Munson (1904) made a detailed study of vitellogenesis and believed that albuminous yolk developed in the subcortical and medullary regions. He also recognized the importance of the yolk nucleus in vitellogenesis and expressed the opinion that formation of the yolk nucleus was due partly to cytoplasmic and partly to nuclear activity.

Trinci (1905) described three kinds of cells in the membrana granulosa of Lacerta muralis and Anguis fragilis. He assigned secretory and nutritive functions to these cells and showed that they send out prolongations into the oöplasm through the zona radiata. He further believed that follicular cells were derived from indifferent cells.

Loyez (1905, 1906) also found three kinds of cells in the egg follicle of certain lizards, snakes, testidunates and crocodiles. The larger cells are rich in nutritive material which passes steadily into the egg, where yolk formation starts in the peripheral and medullary regions. She also showed that the so-called yolk nucleus contained the centrosome in its interior.

Dustin (1910) worked on the derivation of the gonocyte in Chrysemys picta marginata. His conclusions were in general agreement with his earlier work on amphibians (1907) and he demonstrated that in Chrysemys picta marginata three groups of germ cells succeed each other during the developmental history. Jordan (1917) traced in great detail the history of the germ cells in the loggerhead turtle, Caretta caretta.

In 1918, Thing discussed the morphological and functional significance of the zona pellucida in the testidunate eggs. She concluded that the zona pellucida is derived from the follicular epithelium and is composed of inter-cellular substance and terminal bars.

Bulliard (1924), working on Emys lutaria, showed that during the growth phase of the oöcyte mitochondria become scattered in patches and form fat bodies. He completely ignored the existence of Golgi bodies and the part they play in the process of yolk formation. He is, perhaps, the only worker in the field who believes that mitochondria form fat bodies.

Guthrie and Robertson (1925) described the yolk bodies in lizard eggs based on developing ova of Sceloporus undulatus and discussed the transformation of "unsaturated" into "saturated" yolk particles and their characteristic arrangement in the sub-pheripheral zone. They showed that increased formation of mitochondria is followed by deposition of "neutral" fat and "unsaturated" yolk; and finally, by transformation of "unsaturated" yolk and direct elaboration within the cytoplasmic emulsion, the "saturated" yolk is formed. They interpreted cytoplasmic inclusions of the developing oöcyte as intermediate stages and end products in the metabolism of food storage.

In 1925, Bhattacharya gave a detailed account of oögenesis in the Indian turtle, Testudo graeca, and the lizard, Uromastix hardwicki. He traced the Golgi bodies and mitochondria

from their earliest stages to their final role in vitellogenesis. He concluded that proteid yolk is formed by the mitochondria. He also observed the phenomenon of nucleolar extrusions but thought that the extruded nucleoli do not play any significant part in vitellogenesis. He discussed the migration of the follicular Golgi bodies into the oöcyte through the "channel-like processes."

Dutta and Asana (1928), working on the eggs of the lizard, Calotes versicolor, believed that follicular Golgi bodies pass down into the oöplasm through the zona radiata and reinforce the Golgi elements of the egg proper, and that fatty yolk arises from the Golgi bodies and proteid yolk from the mitochondria.

Bhattacharya and Lal (1930) working on the Indian tortoise, Kachuga smithii, came to the conclusion that the fatty yolk is produced by transformation of the Golgi bodies and that the proteid yolk is formed through the intervention of mitochondria. Their investigation gave further evidence in support of Bhattacharya's discovery (1925) on the infiltration of follicular Golgi bodies into the oöcyte.

Lal (1933) worked on four Indian snakes, Zamenis mucosus L., Gongylophis conicus Schneid., Tropidonotus stolatus L., and Tropidonotus piscator Schneid., and confirmed the work of Bhattacharya and Lal (1930). He did not observe the emission of nucleolar material from the nucleus into the egg

cytoplasm. His work was based primarily on the study of fixed material.

Rahn (1938) studied the post-ovulatory changes in the Graafian follicles of seven species of viviparous and two species of oviparous snakes, the horned-toad lizard, and the snapping turtle, revealing the formation of luteal tissue in these reptiles. Cieslak (Unpublished, 1938), describing the seasonal changes in the reproductive organs of the common garter snake, Thamnophis radix,does not mention the presence of a corpus luteum in this snake. However, at about the same time, Dutta (Unpublished, 1938) showed the presence of a corpus luteum and gave a detailed account of the histology of the ovary and testes at different stages of the sexual cycle in the following reptiles: Crocodilus palustris, Gavialis gangeticus, Emyda granosa, Trionyx gangeticus, Hemidactylus fluviviridis, Uromastix hardwicki, Varanus benghalensis, Ptyas mucosus, and Naia tripudins. He also described the common cytoplasmic inclusions in the eggs of these reptiles. Similar studies pertaining to the placentation and corpora lutea in certain European and Australian snakes and lizards were undertaken by Weekes (1934, 1935).

Among the later contributions to the cytological and histological study of the ophidian ovary, mention may be made of the work of Mathur (Unpublished, 1940) on Eryx johnii, a species very closely allied to Eryx conicus. He has described the Golgi bodies as arranged on the rims of fatty vacuoles of the eggs in Eryx johnii. One of his students,

Nand (1944), has worked on the snake Zamenis diadema and made a study of the Golgi elements, but he failed to find the earlier stages of these inclusions.

Risley and Barnett (1941) studied the asymmetrical features of the urogenital system in both the sexes of garter snakes by following the origin and migration of primordial germ cells. They found that the largest number of primordial germ cells occur on the right side in snakes and the primary asymmetry is established early during the stages of migration and formation of the genital ridge. They also concluded that the migration of the primordial cells in Thamnophis spp., occurs mainly through blood vessels, as is the case in Sphenodon (Tribe and Brambell, 1932).

Recently, Srivastava (1947) has worked on five Indian lizards, Hemidactylus gleodovii, Lygosoma punctatum, Calotes versicolor, Varanus griseus, and Uromastix hardwicki. He observed the transmission of the Golgi bodies from the follicular epithelium to the oöplasm in three different ways. They were seen passing in the form of large isolated bodies; as groups of granules through bridges; and some through definite canalicular passages. The mitochondria, in a similar fashion, were seen to infiltrate through narrow and definite bridges.

MATERIALS AND TECHNIQUES

Four ophidian types were selected for this study, namely: three Indian snakes, Natrix piscator piscator (Schneider), Lycodon aulicus aulicus (Linnaeus), and Eryx conicus (Schneider);[1] and one American snake, Thamnophis ordinatus ordinatus (Linnaeus).[2]

Living specimens of Eryx conicus were procured from Gwalior, Central India, and its near vicinity. The specimens of Natrix piscator piscator and Lycodon aulicus aulicus are abundant in and around the Districts of Agra and Cawnpore, the United Provinces, India, and were collected from these localities. The specimens of Thamnophis ordinatus ordinatus were collected near Ann Arbor, Michigan, and the Douglas Lake Region, Cheboygan County, Michigan. In general, snakes are hardy and can be kept alive in the laboratory for long periods but such material was not used. In all cases, the animals were killed soon after capture. The specimens were collected throughout the year except in winter, when the snakes are in hibernation and are difficult to obtain.

The animals were killed by decapitation as soon as they were brought into the laboratory, thus avoiding any changes

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[1] Smith, Malcolm A. The Fauna of British India, Ceylon and Burma. Reptilia and Amphibia. III. Serpentes. London: Taylor and Francis, 1943.

[2] Klauber, L. M. Some misapplications of the Linnaean names applied to American snakes. Copeia, 1:1-14, 1948.

as a result of captivity. Specimens of different age groups were selected for study of the structural condition of the ovary in different stages of growth. The ovaries were removed from immature, ripe, and post-partum females and were quickly transferred to the appropriate fluid for fixation. In general, the fixatives were used in accordance with Gatenby's Vade-Mecum with slight modifications suggested by Dutta (MS, 1938). It was found necessary in this investigation, however, to reduce the proportion of neutral formalin to avoid shrinkage of the oöcytes, which was especially noticeable in the larger ones.

Bouin's picro-formol was employed for material to be used for studying the general microanatomical and histological structure of the ovary and the nucleus, as well as, for following the nucleolar history. It yielded very satisfactory results. Staining was done in Mann's methyl blue eosin, methylene blue eosin (5% aqueous eosin, and 0.1% aqueous solution of methylene blue), Ehrlich's hematoxlin, and Delafield's alum hematoxylin. Heidenhain's iron hematoxylin was also used but did not give very satisfactory results.

For the demonstration of the Golgi bodies, small pieces of ovary were fixed according to Da Fano's cobalt nitrate silver method, Cajal's uranium nitrate silver method, Aoyama's cadmium chloride silver method, and Das' technique of combination of Da Fano's and Cajal's methods. In all the above mentioned fixatives the material was fixed for 24 hours. Later it was given a few quick washes in distilled water and transferred

to 1.5% solution of silver nitrate for 48 hours. Again it was quickly washed in distilled water before being transferred to the Cajal's reducer for 24 hours. This was followed by dehydration and embedding. Staining was not considered necessary but the sections were toned with 0.2% gold chloride solution and later fixed with 5% hypo solution.

The other and more successful technique employed for the study of the Golgi bodies was Ludford's modification of Mann-Kopsch's method. The material was fixed for 18 hours in Mann's fluid:

saturated solution of corrosive sublimate in 0.75% salt solution . . . . . 1 volume;

aqueous solution of 1% osmic acid. . . . . 1 volume.

The material was later washed in distilled water for half an hour and left in 2% osmic acid in a stoppered dark glass bottle for three days. It was given subsequent washes in distilled water to promote satisfactory impregnation. Generally, the ovarian tissue was kept in distilled water for 18 hours, after which it was dehydrated and embedded. The post osmication period was controlled and could not be extended beyond 3-4 days, as the material tended to become brittle and thereafter difficult to manipulate. Staining was avoided in this case too. However, some sections were bleached with 1% solution of potassium permanganate and 4% solution of oxalic acid (Henneguy's method) to take away the excess osmic acid. The slides were mounted in neutral balsam or clarite, and were left to dry in a drying oven.

Different mitochondrial techniques such as Regaud's method, Flemming's method without acetic acid, and Zenker's method were employed in order to distinguish mitochondria from the Golgi bodies and other cytoplasmic inclusions. However, this material was not studied extensively considering the fact that there is general agreement among cytologists with regard to the morphology and physiological role of mitochondria in the growing oöcyte. Mitochondria can now be collected by centrifugation and subjected to direct chemical analysis (Bensley and Hoerr, 1934; Claude and Fullam, 1945).

In supravital staining the fresh ova were examined in strong artificial light, (1) stained with neutral red for the demonstration of vacuome, (2) treated with osmic acid for the demonstration of the Golgi bodies, or (3) stained with Janus green B for the mitochondria. Janus green B and neutral red solutions were always freshly prepared.

An attempt was also made to show Golgi bodies side by side with the vacuome. The ovarian pieces were first stained in neutral red solution, and soon after the appearance of the vacuome 2% osmic acid was introduced under the cover slip. The Golgi bodies took up a black-brown color, while the vacuome remained red (Bhattacharya and Das' method, 1929). The solutions were kept in an electric bath at a temperature of 38 degrees centigrade for a period of 24 hours for ripening. They were subsequently allowed to cool down for the same length of time. These solutions were diluted before before use in the ratio of 12 drops of each solution to 25 cc. of Ringer's salt solution.

The freezing microtechnique was employed for the detection of fat. The material was fixed in Aoyama's fluid for 24 hours and after silverization and reduction it was frozen with compressed carbon dioxide. The sections were cut 20-30 μ thick and were stained in Sudan IV after keeping them for 5-10 minutes in 50% alcohol. The sections were again placed in 50% alcohol after staining. Later, they were downgraded to distilled water. Finally, they were stained with hemalum and mounted in glycerine. The fat globules appeared deep orange and the albuminous yolk violet after this technique.

The ovarian pieces were centrifuged for different periods in an electric centrifuge at a speed of 3,250 revolutions per minute to get distinct stratifications of the various cytoplasmic inclusions and the resultant structures. It found that centrifugation for a period of 3 hours was sufficient to give satisfactory stratifications. After centrifugation the material was immediately fixed according to Ludford's modification of the Mann-Kopsch method and Flemming's technique without acetic acid. The rest of the procedure was the same as described earlier.

The material was sectioned with a rocker or rotary microtome into 4-6 μ thick sections. Photomicrographs were taken on 4 x 5 Kodak M plates. The exposed plates were developed in Eastman D-19 developer and were fixed in hypo solution. Printing was done on AZO F-2, F-3, and F-4 photographic paper.

NATURAL HISTORY OBSERVATIONS

All the snakes employed in this investigation are non-poisonous. One of the four snakes investigated, Eryx conicus, belongs to the family Boidae. The remaining three, Natrix piscator piscator, Lycodon aulicus aulicus and Thamnophis ordinatus ordinatus, are members of the Lycodontine-Natricine sub-family complex of the family Colubridae. These snakes were selected to include representatives of different habits and different habitats.

The genus Eryx includes seven species of which only two are represented in India. Eryx conicus is somewhat restricted in numbers and in its distributional range in comparison to Eryx johnii johnii. The former bears general resemblance to the pythons in habits and appearance. It possesses a conical, short tail, hence the specific name. It is a fairly common snake and reaches a length of 20-24 inches. It can be readily distinguished by means of its peculiar coloration, which is brownish-grey with yellowish-brown blotches on the dorsal side forming a design of an irregular chain. In young and freshly captured specimens coloration is brilliant but in captivity it becomes dull. The projected snout and the inferior mouth are, perhaps, adaptations to its burrowing habit in sandy soil, which it always prefers. It lives in shallow holes and the specimens can be collected by digging in the garden and other similar places. It is rather

a sluggish snake and for this reason makes an excellent pet and is a favorite item of display in a snake-charmer's basket.

Eryx conicus occurs on the plains of India northeastward to Bihar and Orrisa and westward to Sind and Baluchistan. It extends into the Nainital District of the Himalayas but is rarer in South India. It is especially abundant at places where rainfall is heavy and luxuriant forest growths occur. It feeds on small mammals such as mice and squirrels and on frogs. It kills its prey by constriction before swallowing.

Lycodon aulicus aulicus occurs throughout India. It is common near houses. It lives in the crevices of loose brick-work of the many dilapidated buildings in the old and historical town of Agra, U.P., where the collection was made. It is strictly nocturnal, very active and fierce.

Natrix piscator piscator, like all other Eurasian Natricine snakes, differs from the American Natricine snakes in only a few details. It reaches approximately a length of 36-40 inches and is riparian in habitat. It is commonly found distributed throughout India in the vicinity of water such as streams, tanks, ponds and paddy fields. It is a very vicious snake, fiercely active, capable of making a jumping movement on the ground and is a good swimmer. It feeds on frogs and fishes. It does not constrict its prey like Eryx conicus, which has a rather small gape, but swallows it at once. The best time for collecting this snake is when the monsoon is about to break, as it is less active during the hot months.

Thamnophis ordinatus ordinatus is one of the most common and best known snakes in northeastern North America. It prefers to live in low unimproved ground and is commonly found near human habitation. It reaches a length of 24-28 inches or more.

The secondary sexual characters in snakes are not pronounced and for this reason some difficulty was experienced in sexing them in the beginning, especially sexually immature specimens. Later, a successful sexing method was developed. This involved the artificial eversion of the hemipenes in the males and demonstration of their absence in the females.

Not only do these snakes differ in their habits and habitats, but they also differ in their breeding habits. Eryx conicus and Thamnophis ordinatus ordinatus are ovoviviparous and the remaining two are oviparous. Unfortunately there is no standard usage of these terms and various workers have used the terms oviparity, ovoviviparity and viviparity with different shades of meaning. Rahn (1939), for example, applies the term viviparous to certain species of the genera Thamnophis and Storeria. Under the prevailing confusion with regard to the usage of these terms, it has seemed best to use non-technical terms such as egg-layers for oviparous and live-bearing for viviparous forms, in which sense these terms have been used in this investigation.

A collection of Eryx conicus made on February 10, 1944, two miles north of Gwalior City, Gwalior State, India, was

shipped to me at Cawnpore, U.P., India, where the specimens were observed in captivity. On August 5, 1944 an adult female, 536 mm. total length, 122 mm. tail length, gave birth to a litter of ten, which measured 145, 145, 148, 149, 149, 153, 153, 153, 154, 156 mm. Again, on August 12, 1944 another female, 512 mm. total, 101 mm. tail, gave birth to 8 young: 144, 145, 145, 146, 148, 150, 151, 151 mm. The act of birth was not observed.

On April 7, 1947 one female specimen of Thamnophis ordinatus ordinatus with a total length of 850 mm. was captured near Ann Arbor, Michigan. It was killed and preserved on August 2, 1947. It contained nine developing embryos in the right oviduct and four in the left oviduct. The embryos were in early stages of development.

Lycodon aulicus aulicus lays usually 6-8 eggs in a clutch averaging in greatest length 26 mm. Natrix piscator piscator is more prolific and produces 30-40 eggs in a clutch; the eggs average 22 mm. in maximum length.

OBSERVATIONS

General Structure of the Ovary

The following description of the ovary in the snakes investigated is applicable to all four species unless otherwise stated. The ovary is a beaded, thread-like structure, in adaptation to the serpentine body form. The right ovary is situated slightly anterior to the left ovary, as is also the case with the right testis and the right kidney. This fundamental asymmetry has been studied in the embryos of several species of Thamnophis by Risley and Barnett (1941), who have concluded that the asymmetry was established during the migration of the primordial germ cells and thus takes its origin at the time of formation of the genital ridges. No satisfactory explanation other than accommodation to body form has been advanced for this asymmetry. I have found that generally the right ovary is more actively functional and produces more ova than the left. One female of Thamnophis ordinatus ordinatus examined had nine developing embryos in the right oviduct and four in the left. However, in this particular case there is no evidence to suggest that all the embryos in the right oviduct were necessarily derived from the right ovary. The heightened activity of the right ovary in snakes is a unique feature. In birds, which have evolved from reptiles, the condition is reversed; only the left ovary and the left oviduct develop whereas those on the right side atrophy.

Along the beaded ovaries run the slightly coiled oviducts. The oviducts on both sides open independently into the cloaca, posteriorly. Running parallel to the ovaries and oviducts there is also a major blood vessel, which branches to form a capillary network around them. The network of capillaries indicates that there is an abundant exchange of metabolic materials. Such a high degree of vascularization cannot be without significance. Histological sections of the ovary show blood vessels adjacent to the follicles (Fig. 8). Through these blood vessels large quantities of raw materials are supplied which must be essential for the growth of the oöcyte. The extensive blood supply continues even after ovulation in connection with the development of the corpus luteum. In Thamnophis ordinatus ordinatus the embryos during their development in the oviduct receive a rich vascular supply. The oviduct at this stage becomes very thin and membranous.

The ovary is supported and suspended in the general body cavity by a mesovarium, which consists of connective tissue covered by an extremely thin and delicate epithelium. Ova are found in different stages of development embedded in the ovarian stroma. Their location bears no relation to their size or state of development. Among the larger oöcytes are found other oöcytes of varying sizes. This suggests that the younger oöcytes can undergo their development unhampered by the presence of the more advanced ones. It appears, therefore, that there are several generations of ova undergoing development simultaneously.

The shape of the ova in Eryx conicus, Natrix, piscator piscator and Thamnophis ordinatus ordinatus continues to be more or less spherical even in later stages, but in Lycodon aulicus aulicus an ovoid form is assumed in comparatively early stages. In young and immature females the ova are more uniform in size than in mature adults.

The ovary is covered by an adipose material in the form of fat bodies. The amount of fat seemingly remains the same even after hibernation, which suggests that it is not used for nutritional purposes during this period. A more probable explanation of its function, as indicated by Volsöe (1944), is that it helps in maintaining the body heat of snakes during hibernation.

Histologically the ovary is a hollow structure enclosing a cavity which is referred to as the ovocoele ("Lymphräume" of Hoffman, 1890) in this paper. In general, the ovary has a very limited amount of stroma. The germinal epithelium, which covers the outer surface of the ovary, is more prominent in immature and young specimens. It has two kinds of cells, namely, the oögonia, which are large and rounded cells with prominent nuclei, and the epithelial cells, which are smaller and slightly elliptical, with less prominent nuclei (Fig. 1). The chromosomes of the more advanced oögonia are conspicuous but counts were not made. The origin and formation of these two kinds of cells in the germinal epithelium was not determined, since the early embryology was not studied. The fact that most of the larger

ova are found in the middle part of the ovary suggests that the mid-portion of the germinal ridge is relatively more active or at least more effective in forming new ova than are the two ends.

In general, the structural condition of the ovary depends on the season of the year and the stage of maturity of the individual. When the eggs are laid, or the young are born, the germinal epithelium is very thin. Immediately after this stage, an increase in thickness due to repeated mitoses takes place. This multiplication period is followed by growth of the primary oöcytes. Before the animals hibernate, the eggs have seemingly reached their full size. This crop of ova is apparently ready for ovulation in the spring, and copulation takes place soon after emergence from hibernation. A feature of the growth period is the fact that some oöcytes enlarge, while others fail to grow. This may be explained as a result of physiological competition for available nutritional materials. A good illustration of differential growth of the oöcytes during this period is seen in figure 6. After the initiation of the growth period, the mitotic activity of the germinal epithelium gradually decreases. Since several size generations of ova are present it appears that it takes more than one year for each generation to develop.

Although the general history of the chromosomes during oögenesis was not worked out in detail, some of the characteristic stages were observed such as the resting stage, the leptotene, the synaptene, the pachytene, and the early and

late diplotene stages. Throughout these stages of the first maturation division, the coiled spireme is a very prominent structure (Fig. 2). The occurrence of atypical oöcytes showing a polynuclear condition was observed. In figure 3 a binucleate oöcyte is shown.

During growth of the oöcyte, epithelial cells constituting a follicle come to surround it. As the oöcytes enlarge, they protrude into the hollow cavity of the ovary, the ovocoele, where they undergo further growth. The oöcytes in the ovocoele are supported by a mesentary through which they are supplied by blood vessels. The ovocoele is filled with a fluid, which serves two possible purposes, namely, to act as a shock-absorbing cushion for the ova lying in the ovocoele, and to provide nutrition for growing oöcyte supplementary to that absorbed through blood vessels.

Egg Membranes

The follicle in the advanced oöcytes of all the snakes investigated is a well differentiated structure. It consists of a zona radiata, a three layered follicular epithelium, and an outer theca layer. The structure of a fully developed egg follicle is shown in figure 7. The zona radiata is not differentiated into striated and homogeneous portions, as described for some reptilian eggs. It consists of a fairly broad, non-cellular layer with distinct radial striations. Next to the zona radiata is the limiting or vitelline membrane of the oöcyte which, however, becomes inconspicuous

and finally disappears or merges with the zona radiata in later stages of growth. A thin portion of the peripheral cytoplasm of the oöcyte becomes highly vacuolized and forms a distinct layer. This vacuolar layer may be interpreted as corresponding to the cortical layer of fibrillae described by Gatenby (1922b) in the eggs of Ornithorhynchus paradoxus, and the fibrillar layer described by Bhattacharya (1925) in the eggs of Testudo graeca.

Lycodon aulicus aulicus provides the best material for the study of the egg membranes and the entire development of the follicle has been traced in this snake. The earliest oöcytes are without any visible enveloping membranes aside from the cell membrane (Fig. 1 and Fig. 2). During growth of the oöcyte, the presumptive follicle cells which are scattered irregularly among the oöcytes in the germinal epithelium become very active and migrate around the oöcytes. This activity is illustrated on the left margin of figures 2 and 3. The disposition of the presumptive follicle cells and their relationship to the developing oöcyte is also shown. In figure 2 only a few follicle cells are seen arranged around an enlarged oöcyte. Figure 4 shows the complete arrangement of these cells around an enlarged oöcyte. However, at this stage the follicle cells are not arranged into a definite layer. The process of migration and multiplication continues and finally the follicle cells become organized into a primary follicle. The primary follicle soon changes from a single-layered condition to a double-layered

one (Fig. 5). Thereafter, the cells of the follicle multiply and differentiate into the several layers described previously and shown in figure 7. An intermediate stage is shown in figure 6. Here the follicle cells are multiplying but they have not yet differentiated into any of the characteristic cell types of the mature follicular epithelium.

The follicular epithelium is perhaps the most significant part of the follicle in view of the fact that it acts as a depot of nutritive materials constantly elaborated by the follicular cells and passed into the oöcyte. These nutrients must play a vital part in the growth of the oöcyte. The usual appearance of the Golgi apparatus and nucleoli in cells of the follicular epithelium indicates that these cells are in an active metabolic condition.

The follicular epithelium has three layers of cells. The cells of the middle layer are conspicuously large and somewhat spindle-shaped. They are perhaps secretory in function and are involved in the elaboration of vast quantities of nutritive materials, which will eventually be passed into the growing oöcyte. The small rounded cells of the inner and outer layers become very active after ovulation. They multiply rapidly and fill the space left by the oöcyte. In this way a corpus luteum is formed. The theca layer supplies supporting connective tissue fibers and capillaries. This structure bears a striking resemblance to the corpora lutea of mammals (Fig. 9). Conflicting views are held with regard

to its function. Further research is needed to determine its structural and functional homology to the corpus luteum of mammals.

The zona radiata, as has been suggested by Gatenby (1922b)and others, has a two-fold function. Firstly it acts as a convenient medium for the passage of follicular Golgi bodies and other cytoplasmic materials into the oöplasm and secondly it unites with the vacuolar layer to form a protective covering, thereby assuming the initial function of the vitelline membrane of the oöcyte which gradually disappears. The fact that mature oöcytes offer greater resistance to puncture and diffusion of stains lends support to the view that the zona radiata assumes a secondary protective function in later stages.

In summary, it may be stated that different parts of the follicle play distinct but integrated roles in the growth of the oöcytes. The oöcyte has inadequate intrinsic resources and must depend on outside sources for a supply of nutrients for its growth. Overwhelming evidence shows that this need of the growing oöcyte is met by the follicle. The zona radiata has a dual function as already described. The follicular epithelium contributes Golgi bodies and other inclusions to the developing oöcyte. Once the nutritional demands have been met in such a remarkable manner the follicle changes into a corpus luteum which perhaps assists in the retention of intra-uterine eggs or embryos.

Nucleolus and Nucleolar Extrusion

Two kinds of nucleoli, namely, the "true nucleoli" or plasmosomes and "false nucleoli" or karyosomes, have been studied in this investigation. The terminology given by Smith et al (1940) was found satisfactory and has been followed in differentiating these two kinds of nucleolar bodies. As is pointed out later, these bodies have been found to be structurally different in this study. A separate account as to their nature and function is included below.

The oögonia and early oöcytes in the germinal epithelium possess a prominent nucleus. The nucleus is relatively large and occupies a major portion of the cell, cytoplasm being restricted to a thin peripheral rim. As already observed, the chromatin material during the early stages of maturation division is in the form of a prominent spireme. There is no indication of the presence of a nucleolar body at this stage. Later, the nucleus appears filled with a loose reticulum of achromatic strands in the meshes of which are suspended chromatin material and a single plasmosome (Fig. 6). Staining with methlylene blue and eosin shows it to be basophilic. This structure is regarded as being homologous to the plasmosome of Smith et al (1940), even though these authors define the plasmosome as being acidophilic. The plasmosome in the early stages of its formation appears homogeneous and does not show any signs of vacuolization. It may also be noted that in the early stages it is invariably the plasmosome which is seen, the karyosome does not appear until later.

In advanced oöcytes all traces of the chromatin material are lost to view (Fig. 10) and the plasmosome becomes a prominent structure inside the nucleus. The nucleus leaves its initial central position, becomes reduced in size and comes

to lie near the surface of the oöcyte. Commensurate with the growth of the oöcyte, the plasmosome grows to a considerable size and soon begins to bud off several smaller bodies. The budding process of the plasmosome is shown in figure 11. It can be seen still better in figure 12, where several such bodies are being formed. These daughter plasmosomal bodies are generally of the same size, but exceptions to this may be found. The daughter plasmosomal bodies come to lie near the nuclear membrane and are eventually extruded into the cytoplasm. It may be mentioned that extrusion of plasmosomal bodies does not last throughout oögenesis. Also there is a considerable variation in the form and size of the plasmosome even in adjacent oöcytes.

The extrusion of the plasmosomes is convincingly shown in figure 13 and 14. A linear series of about a dozen extruded plasmosomes may be seen in figure 13. These extruded plasmosomes become scattered irregularly in the cytoplasm. Figure 14 indicates the manner in which the plasmosomes pass out into the oöplasm. Here one plasmosomal body lies half inside and half outside of the nucleus. In addition, this figure shows three plasmosomal bodies in the oöplasm and one in the nucleus. The staining nature of the extruded plasmosomes and the plasmosome inside the nucleus is the same. It appears that the plasmosomal bodies gain exit through breaks in the nuclear membrane. The recovery of the membrane from the break is apparently rapid and complete.

Figure 15 shows the eccentric position of the nucleus in an advanced oöcyte. In this figure one plasmosomal body is shown in the process of migration toward the peripheral region of the oöcyte. It lies midway between the egg nucleus and the follicular epithelium. A few plasmosomal bodies which have not yet migrated into the oöplasm are shown inside the nucleus. Attention is called to the vacuoles surrounding the plasmosomal bodies inside the nucleus. These spaces are common especially in the nucleus of Eryx conicus. It appears that some of these bodies pass out into the cytoplasm immediately after their formation, and that in other cases they are retained in the nucleus for sometime during which vacuoles develop around them.

The plasmosomes are negative to the Feulgen reaction and have, therefore, no chromatin constituents. They are basophilic as a rule, but in several instances they were also acidophilic. This variation in the staining capacity of the plasmosomes has been reported by earlier workers and has been individually interpreted by them. A full discussion on the staining reactions of the plasmosomes will follow later. Variations in staining capacity of these bodies is a feature which should not be overlooked.

In figure 12 the much enlarged plasmosome is shown to be differentially stained. The central part is deeply stained, while the outer part is lighter. This difference may be interpreted as due to a variation in the concentrations of materials present in it rather than to its dual

nature. Figure 12 is interesting from another point of view as well. It shows in a clear manner the formation of daughter plasmosomal bodies from the principal plasmosome and indicates that these daughter plasmosomal bodies are not formed from any other source, such as through the agency of plasmosomal organizers which might be present in the chromatin of the nucleus. There is no increase in size of the plasmosomal bodies after they reach the cytoplasm, neither do they multiply. They completely dissolve in the oöplasm without being directly transformed into any kind of yolk material. It is suggested that extrusion of the plasmosomal bodies provides the cytological mechanism for the exchange of certain activating chemical substances from the nucleus to the cytoplasm. Presumably, these activating chemical substances are essential for the elaboration of yolk material.

The above account concerns the plasmosome and its derivatives. The account which follows pertains to the karyosome, the second kind of nucleolar body found in the oöcyte. Figure 16 brings out clearly the different nature of these two types of nucleoli. In this figure several small plasmosomes and a large karyosome are shown scattered in the cytoplasm. The karyosome lies between the egg nucleus and the follicular epithelium. Figure 19 shows the same karysome under a higher magnification. It appears like a miniature nucleus and possesses chromatin material. It is not homogeneous like the plasmosome. It seems logical to conclude that this Karyosome has been extruded into the cytoplasm from the egg nucleus.

Figure 17 shows karyosomes inside the egg nucleus lying in spaces similar to those found in the case of plasmosomes. A karyosome is shown in figure 18 pushing the nuclear membrane in the process of being extruded, however, the extrusion of karyosomes is less frequent.

No suggestion can be offered at present as to the functional significance of the extruded karyosomes. The karyosome is regarded by some workers to be a storehouse of basichromatin. The follicular epithelium also contributes chromatin material to the oöplasm during oögenesis as described below.

Emission of Follicular Nuclei

The emission of chromatin material into the cytoplasm from the egg nucleus in the form of karyosomes has already been described under the heading Nucleolus and Nucleolar Extrusions. In one of the snakes investigated, namely, Lycodon aulicus aulicus follicular nuclei and, at least in some cases, the entire epithelial cells were observed to migrate into the oöplasm from the follicular epithelium. So far as I can ascertain, this has not been observed before. Although there are several references in the literature to the presence of chromatin material in the oöplasm during oögenesis the source of this extruded chromatin has been invariably traced to the egg nucleus. Figure 20 shows two follicular nuclei lying in the oöplasm. These extruded nuclei resemble in a striking manner the nuclei inside of the follicular epithelium and their close proximity to the

follicular epithelium indicates clearly that they are derived from the follicular epithelium.

The extruded nuclei tend to enlarge inside the oöplasm. For instance in figure 20 one of the extruded nuclei is much larger than the rest. Eventually these extruded nuclei are dissolved and their contents are diffused in the cytoplasm, leaving no trace of their presence.

The exact significance of this extruded chromatin material is difficult to determine at present. The demonstration that the nuclei of some cells do migrate to take part in the nutritive or vegetative functions of a growing cell such as the oöcyte appears to be of importance.

Golgi Apparatus of the Oöcyte

The considerable difficulty experienced in identifying the Golgi apparatus is recognized in this investigation. It is necessary to differentiate Golgi material from two other cytoplasmic constituents, namely, mitochondria and fatty yolk. In the osmic preparations the Golgi bodies could be differentiated from fatty yolk bodies by differences in size, time interval required for impregnation, solubility in fat solvents, and staining capacity with Sudan IV. The possibility that some of

these granules may be mitochondrial in nature was precluded by bleaching these preparations and staining them with acid fuchsin. These bodies did not take the stain and it is, therefore, logical to conclude that they were not mitochondria. The silver preparations were also helpful in differentiating these structures because, as a rule, the mitochondria and fatty yolk bodies are not fixed in these preparations and only the Golgi bodies are shown.

Some of the oöcytes in the germinal epithelium show a distinct outline, while others are so crowded that their outline is not clearly visible. An oöcyte with a distinct outline can be seen at the upper portion of figure 22. At this stage the nucleus is the most prominent cellular component. The cytoplasm appears as a thin rim surrounding the nucleus. The nucleus contains a prominent plasmosome which stains yellowish in osmic preparations.

In early oöcytes the Golgi apparatus occurs as a compact mass of minute osmiophilic or argentophilic granules situated in juxtanuclear position. In the earliest stages the Golgi apparatus consists of only a few granules but it becomes more dense commensurate with the growth of the oöcyte. Figure 21

shows the dense juxtanuclear mass of the Golgi bodies in an early oöcyte. It is apparent that this part of the oöplasm is different from the rest and is distinguished as the "yolk nucleus area." Seemingly the "yolk nucleus area" is the center of cytoplasmic activity.

In figure 22 there is a noticeable increase in the bulk of Golgi bodies and the juxtanuclear mass has assumed an eccentric position. In still later stages of growth of the oöcyte, the Golgi bodies appear as a perinuclear ring. In figure 23 several oöcytes are shown having the circumnuclear concentration of the Golgi bodies. The entire cytoplasmic rim is filled with them. The perinuclear stage persists for some time.

Figure 24 shows an advanced oöcyte. Although the Golgi bodies have at this stage increased both in size and in number and are about to be dispersed in the cytoplasm, their original juxtanuclear position is still retained. Figure 25 shows a still later stage in the dispersal of the Golgi elements. Here they are scattered in the cytoplasm. The relatively less dense central area suggests that the Golgi material from this area has already migrated toward the periphery. During the dispersal stage, the Golgi bodies show an appreciable increase in size and number. Throughout the cytoplasm are scattered disconnected fragments or isolated granules of of Golgi sutstance. The Golgi bodies thus undergo definite topographical changes in an orderly sequence. It is postulated that these changes are brought about by changes in

their polarity. At the present state of our knowledge about this cytoplasmic structure, however, it would be a matter of speculation to suggest what physico-chemical factors govern these changes in the polarity of the Golgi apparatus.

In the next stage the Golgi bodies are seen arranged in a definite row in the peripheral region. Their orderly arrangement, shown in figure 26, is very striking. It persists for a long time and is therefore the easiest to encounter in sections. The peripheral concentration of the Golgi bodies gradually increases (Fig. 27). At about the same time a second source of the Golgi material is added from the follicular epithelium. Thus a thick peripheral concentration of the Golgi bodies is formed (Fig. 32). The exact manner in which the oöcyte received the follicular Golgi bodies is described under the heading Follicular Golgi Bodies below. Later, a redispersal of the Golgi bodies into the interior of the cytoplasm occurs. As indicated before, these progressive changes in the position of the Golgi bodies throughout oögenesis must be due to some kind of physiological reversal in their polarity with respect to other components of the cell. After the redispersal stage the Golgi granules aggregate into larger bodies and are directly transformed into the fatty yolk. Details of the formation of fatty yolk are given under the heading Vitellogenesis. Contrary to the findings of Bhandari and Nath (1930) in the eggs of Dysdercus cingulatus, no indication of any fatty yolk was found in early oöcytes. It is concluded, therefore, that the formation of fatty yolk starts late during the growth of the oöcyte.

There was a remarkable similarity in distributional and structural details of the Golgi bodies studied in both fixed and fresh preparations. The Golgi bodies throughout oögenesis showed a homogeneous granular or spherical structure. At no time were they observed to have a duplex structure with a chromophilic rim and chromophobic core, as described by some workers. In some of the silver preparations, however, a few Golgi bodies were seen as crescent-shape dictyosomes. Such morphological variations from the usual granular type were extremely rare and are interpreted to be caused by heavy silverization. At any rate silver techniques are not regarded ideal for the study of the structure of Golgi bodies, because silver impregnation tends to distort their form. It is concluded that such dictyosomes are probably an artifact.

Follicular Golgi Bodies

As described previously, the follicular epithelium has two distinct types of cells, namely, small, rounded cells and large spindle-shaped cells. All these cells possess a large nucleus which contains a prominent plasmosome. Each cell also contains Golgi bodies demonstrable both by silver and osmic impregnations. The appearance of the follicular epithelial cells indicated that they are in an active metabolic condition.

The Golgi bodies of the follicular epithelial cells are very well developed. They are seen in a juxtanuclear position

in the early stages and may be in separate groups. Figure 28 shows a cell having three clusters of Golgi material in position. A circumnuclear concentration of the Golgi bodies was not often seen. The Golgi bodies soon leave the juxtanuclear position and are dispersed throughout the follicular epithelial cells, following increase in size and number. This stage is seen in figure 29. The large numbers of Golgi bodies present in these cells are considered as an indication of high metabolic activity. Later, they accumulate near the zona radiata as shown in figures 29 and 30.

The most interesting feature in the growth of the follicular Golgi bodies is the fact that they infiltrate into the oöplasm through the zona radiata. Thus a definite quota of Golgi material is supplied to the growing oöcyte by the follicle. This process of infiltration is very clearly demonstrated in figure 30. The entire zona radiata is filled with Golgi bodies which are in the process of being transferred to the oöcyte. In the same figure may be seen some Golgi bodies crossing over from the follicular epithelium to the peripheral region of the oöcyte. The zona radiata seemingly does not offer any resistance to the passage of the Golgi bodies and infiltration occurs regularly through it.

The infiltration occurs through the migration of isolated bodies. In none of the snakes investigated were there observed any definite channels or bridges for the infiltration of the Golgi bodies. Such definite channels and bridges have been described by some workers in other reptilian eggs.

The Golgi bodies from the follicular epithelial cells may either infiltrate immediately upon reaching the zona radiata or may accumulate there before they are passed into the oöplasm. The infiltrated follicular Golgi bodies come to lie in the peripheral region of the oöcyte where they intermingle freely with the Golgi bodies of the oöcyte. The zona radiata becomes entirely free of the Golgi elements when the process of infiltration is completed. Also the follicular cells show a marked reduction in the amount of the Golgi material. This condition is shown in figure 31.

It was found in this investigation that Aoyama's cadmium chloride silver method and Ludford's modification of Mann-Kopsch method yielded better results than any other techniques for demonstrating the phenomenon of infiltration. The convincing demonstration of this phenomenon in the ophidian egg indicates that the follicle plays an important physiological role in the growth of the oöcyte.

Vitellogenesis

It is a matter of common knowledge that telolecithal eggs at the time of ovulation are endowed with large quantities of reserve food material in the form of carbohydrates, fats, and proteins. The eggs of the snakes investigated in this study are no exception and are full of yolk bodies which are consumed during development of the embryo. Some substances are present in dissolved state and are, therefore, not visible under the microscope.

In all the four snakes investigated, two distinct types of yolk bodies were observed, the fatty yolk bodies and the proteid yolk bodies. Occasionally both kinds of yolk bodies could be seen in the material fixed by Ludford's technique. In such preparations the fatty yolk bodies appear black and the proteid bodies grayish. On staining these preparations with acid fuchsin or iron hematoxylin the proteid yolk bodies are stained pinkish or bluish, while fatty yolk bodies remain unstained. In silver preparations fatty yolk bodies are not observed at all and the only indication of their presence is afforded by empty spaces called the fatty vacuoles.

The origin of fatty yolk bodies has been traced. It has been shown in a conclusive manner that fatty yolk bodies arise directly from the Golgi elements. Immediately preceding the formation of fatty yolk in the oöcyte, a marked hypertrophy of the Golgi bodies occurs. Later, fusion of these hypertrophied Golgi elements takes place resulting in the formation of fatty yolk. In figure 33 some of the smaller Golgi bodies can be seen undergoing hypertrophy. On the right margin of the same figure are seen two transformed fatty yolk bodies. Several intermediate stages in this transformation process can be clearly made out in this preparation. In figure 34, which is a portion of an oöcyte of Eryx conicus, similar stages in the transformation of Golgi bodies to fatty yolk are shown. By a close examination of this preparation one can identify small Golgi granules, aggregated Golgi granules, and the transformed large fatty

yolk bodies. Some of the fatty yolk bodies which are in a transitional stage even show a granular appearance. However, this granular appearance is soon lost and they become homogeneous. The close association of the Golgi bodies and the fatty yolk bodies affords a clue to the origin of the latter by direct transformation, a supposition which is further substantiated by the study of the intermediate stages.

As already mentioned, there are no traces of fatty yolk formation in early oöcytes. It is concluded, therefore, that fatty yolk formation starts relatively late during growth of the oöcyte. Ordinarily, vitellogenesis starts when maximal development of the Golgi bodies in the oöcyte has occurred and a definite quota of the follicular Golgi elements has been supplied. In rare cases, however, yolk formation may be initiated before the establishment of the peripheral concentration of the Golgi elements in the oöcyte. It seems that formation of fatty yolk must be dependent on some physiological requirements of the growing oöcyte and it does not invariably occur in a definite sequence.

The formation of fatty yolk may be seen in any region of the oöcyte, although the usual places are the peripheral region and "yolk nucleus area." In Thamnophis ordinatus ordinatus fatty yolk formation takes place in the peripheral region of the oöcyte (Fig. 35). Notice the inner dense concentration of the Golgi bodies and the outer layer of fatty yolk bodies. In Lycodon aulicus aulicus fatty yolk formation occurs in the "yolk nucleus area" as shown in figure 37.

However, sometimes the fatty yolk formation starts at one end of the oöcyte as shown in the oöcyte of Natrix piscator piscator (Fig. 36).

The fatty yolk bodies dissolve on treatment with fat solvents such as pure turpentine. They leave empty spaces on dissolution, spoken of as fatty vacuoles. Sometimes the poorly fixed fatty yolk bodies are dissolved readily in xylol. It is likely that the degree of dissolution of the fatty yolk bodies is dependent on the amount of free fat present and also on the state of saturation of the fats involved. If the amount of free fat present is large the entire fatty yolk bodies dissolve in turpentine in a short time; likewise they have been seen to disappear in xylol during the preparation of the slides. However, some fatty yolk bodies offer great resistance to the dissolving action of the fat solvents. The failure of turpentine to dissolve some fatty yolk bodies is interpreted by postulating that these bodies have not been completely transformed into true fat.

The fatty yolk bodies were stained deep orange with Sudan IV, both in fresh and formalin-fixed material. However the study of this material did not provide any clue to the origin of fatty yolk bodies. The main reason for this failure may be the fact that the fatty yolk bodies absorb Sudan IV only when they are completely of fatty nature. According to this idea those which were in the transitional stage and contain material other than true fat would, therefore, not absorb the fat-specific Sudan IV.

The origin of proteid yolk was not traced. However, in following the fate of the extruded plasmosomes, the conclusion was reached that the proteid yolk is not formed by direct transformation of these extruded bodies. In the first place, the extruded plasmosomes are numerically insignificant as compared to the number of proteid yolk bodies found in an advanced oöcyte; nor is there any evidence of active multiplication of these extruded plasmosomes in the cytoplasm. Secondly, the extruded plasmosomes did not show any increase in size to reach the dimensions of the proteid yolk bodies. On the contrary, they were seen to dissolve in the cytoplasm thereby diffusing their materials throughout the oöcyte. Following dissolution they are no longer visible under the highest resolving power of the microscope. In snakes, therefore, proteid yolk is not formed by the method of direct transformation of extruded plasmosomes, as described by some workers in the case of certain vertebrate and invertebrate eggs. It is altogether probable, however, that the extruded plasmosomes play an indirect part in building the proteid yolk of the oöcyte.

Some workers have shown in a variety of vertebrate and invertebrate eggs that the proteid yolk is formed by the direct transformation of the mitochondria in a manner somewhat similar to the formation of fatty yolk by the direct conversion of Golgi elements. Presumably, this is true in the case of snakes. However, to bring about this transformation some activating chemical substances are undoubtedly

needed. It is possible that the extruded plasmosomes provide the mechanism for the supply of these essential materials to the oöplasm from the nucleus. A complete treatment of the possible role of the extruded plasmosomes in the elaboration of proteid yolk is given in the Discussion.

Centrifugation

The study of centrifuged eggs is of very great value in establishing the true relationship and separate identity of the various cytoplasmic inclusions. Through the use of strong centrifugal force the visible cytoplasmic materials can be stratified according to their specific gravity into separate and distinct layers. Beams (1943), summing up the importance of centrifugation towards our understanding of the true nature of the Golgi apparatus, writes: "These experiments lend evidence to the view: (1) that the Golgi apparatus represents a definite material within the cell, (2) that in certain cells, at least, it has a lower specific gravity than the cytoplasm in which it is embedded and (3) that after being displaced no evidence marking its former position in the cell is observed."

In this investigation, satisfactory results were obtained by fixing the centrifuged material according to Ludford's modification of the Mann-Kopsch method or Flemming's technique without acetic acid as described under the heading of Materials and Techniques. Three distinct zones were stratified. The lower zone contained the mitochondria and

nucleus. The middle zone, occupying the greatest portion of the oöcyte, contained proteid yolk bodies. The upper portion, in which are segregated the lightest elements, contained the Golgi bodies and fatty yolk bodies. The fact that the Golgi bodies and fatty yolk are stratified in the same layer lends to the view that formation of fatty yolk takes place by direct transformation of the Golgi bodies. Some oöcytes showed the Golgi bodies and mitochondria in the middle zones, perhaps as a result of incomplete stratification.

Supravital Staining

In modern cytology considerable emphasis is given to vital staining, since it has become evident that cellular structures rarely lend themselves to faithful preservation by fixing fluids. Fresh material was used in this investigation to provide confirmation of the results obtained by the study of fixed material described under the heading of Materials and Techniques. There was complete correspondence in the structural details revealed by the two methods. The rapid smear techniques provide a faithful representation of cytoplasmic components and are considerably less time consuming.

(1) Neutral red. Oöcytes were kept in a solution of neutral red for varying lengths of time; a period of 20-30 minutes was considered sufficient for best results. Advanced oöcytes were punctured by a needle to facilitate penetration of the neutral red solution. In general, oöcytes containing

much yolk were avoided, as the deutoplasmic inclusions prevented the study of living cytoplasmic structures. Similarly, very small oöcytes were avoided, as their cytoplasmic inclusions were so minute that they could not be easily identified. In young oöcytes a number of refringent granules, which did not take the neutral red stain, were seen scattered irregularly in the cytoplasm. These refringent granules were identified as Golgi bodies. In addition to these granules, red vacuoles were observed which were also irregularly scattered in the cytoplasm. These red vacuoles were identified as the so-called vacuome. In the early oöcytes the vacuome was seen in close association with the Golgi granules, although it stood out quite distinctly from the latter. In advanced oöcytes the vacuome groups are scattered throughout the cytoplasm in increasing numbers. At this stage their original association with the Golgi granules is lost. The yolk bodies likewise in advanced oöcytes were distinct from the red vacuoles and seemed to bear no relationship to them.

Observations on the Golgi bodies in the material treated with neutral red were made by introducing 2% osmic acid under the coverslip. This treatment rendered the contrast between Golgi bodies and vacuome very distinct. The osmic acid showed the Golgi bodies as highly refractile black-brown granules. The vacuome was not affected by introduction of osmic acid and could still be seen in the form of brilliant pink vacuoles. It is concluded that these two cytoplasmic structures are distinct from each other and are separate entities.

(2) Osmic acid. Attempt was made to study Golgi bodies during their entire cycle of activity by using 2% osmic acid for varying periods of time. In such studies the Golgi bodies were never seen to have a duplex structure. They were invariably in the form of homogeneous granular or spherical bodies. In advanced oöcytes certain larger bodies, which became blackened with osmic acid, were identified as fatty yolk. Other larger bodies which did not blacken with osmic acid but appeared whitish in these preparations were identified as proteid yolk bodies. A clear distinction can thus be made between these two types of yolk bodies. In an advanced oöcyte, Golgi bodies and the two types of yolk bodies can be distinctly identified. There is an evident decrease in the number of Golgi bodies corresponding to the greater elaboration of fatty yolk. This suggests strongly that Golgi elements are directly transformed into fatty yolk.

(3) Janus green B. Janus green B was used to demonstrate mitochondria. Details of procedure were the same as followed in the case of neutral red staining. The mitochondria were stained greenish-blue by this technique and appeared granular. Yolk bodies remained unstained by Janus green B, clearly distinct from the mitochondria.

DISCUSSION

Egg Membranes

The membranes surrounding the eggs of vertebrates have engaged the attention of several workers (Rathke, 1839; Leydig, 1853; Waldeyer, 1870; Eimer, 1872; Loyez, 1905, 1906; Thing, 1918; Gatenby, 1922b; Champy and Gley, 1923; Pincus and Enzmann, 1937; Guthrie and Jeffers, 1938). It is customary to speak of egg membranes as primary, secondary and tertiary, according to their origin. Those which are produced by the egg itself are called primary membranes, such as the limiting membrane or vitelline membrane. Secondary membranes are those which are formed by the surrounding cells of the ovarian stroma or the follicle. Tertiary membranes are those which are formed by the oviduct, such as shell membranes and the shell. This discussion, however, pertains mainly to the secondary membranes.

The egg follicle in snakes is well differentiated and represents an intermediate condition in the evolutionary sequence of this structure in the vertebrate series. In the eggs of fishes, the follicle is generally represented by a few cells. These cells are small and inconspicuous and can be identified principally by the presence of nuclei around the oöcyte. In the eggs of mammals, the follicle finds its maximum development in the form of a Graafian follicle. The intermediate condition found among snakes is of considerable

evolutionary significance. In this study the entire sequence in the formation of the follicle has been traced (Figures 2, 3, and 5). The follicle of a mature oöcyte consists of a zona radiata, a stratified follicular epithelium and theca folliculi (Fig. 7). The zona radiata is not divided into a homogeneous and a striated portion as described by Srivastava (1947) in Hemidactylus gleodovii. He himself did not find this differentiation in other lizards.

The origin of the zona radiata has been the subject of considerable controversy. Some workers have considered it follicular in origin, while others have thought it to be a product of the oöcyte. Thing (1918) showed that in turtles the zona pellucida consists of a homogeneous layer and a system of canals. Similar canalicular prolongations of the follicular epithelial cells in the zona radiata have been previously described in other reptiles by Waldeyer (1870) and Loyez (1905, 1906). Gatenby (1922b) described the following membranes in the advanced oöcyte of Ornithorhynchus paradoxus: a theca externa, a theca interna, a double-layered follicular epithelium, a zona radiata, a cortical layer of fibrillae, and a limiting membrane of the oöcyte. His work, however, did not provide any conclusive evidence regarding the origin of the zona radiata. In the snakes, the zona radiata is formed only after the follicular epithelium has assumed a multi-layered condition. This conclusion is in agreement with the findings of Gatenby (1922b), Brambell (1928), Guthrie and Jeffers (1938) and Kingsbury

(1939). It seems logical to conclude in the light of their work and the evidence provided in this study that the zona radiata is the product of the oöcyte.

The egg follicle in snakes takes part significantly in the growth of the oöcyte. Although the idea that it provides nutritive materials to the egg through the zona radiata was first expressed by Waldeyer (1870) and later by Loyez (1905, 1906), sufficient experimental evidence was not forthcoming to substantiate it until Brambell (1926) in Gallus bankiva demonstrated the infiltration of follicular Golgi elements into the oöplasm. Later, the infiltration of follicular Golgi bodies and mitochondria was observed by Dutta and Asana (1928) in Calotes versicolor; Bhattacharya, Das and Dutta (1929) in certain fishes, amphibians, reptiles, and birds; Lal (1931, 1933) in snakes; Clement (1933) in squirrels; and Srivastava (1947) in lizards. In the snakes, infiltration of Golgi bodies occurs as isolated granules and not in the form of linear chains as described by Srivastava (1947) in the eggs of Hemidactylus gleodovii and Lygosoma punctatum. However, he found in three other lizards investigated that the infiltration does take place in the form of isolated granules. It is clear from the evidence provided by my study as well as by a survey of the literature that infiltration of follicular Golgi bodies is an established phenomenon in the vertebrates.

In the present investigation extrusion of the follicular nuclei into the oöplasm has been demonstrated for the

first time. There are, however, several instances in literature where chromatin material has been reported in the oöplasm during oögenesis but its source has been invariably traced to the egg nucleus. I have demonstrated in the case of snakes that nuclei of follicular cells come out into the oöplasm, perhaps, to take part in the nutritional activities of the growing oöcyte. In my opinion, it is likely that the extruded follicular nuclei represent trophochromatin specialized for a vegetative function. Extrusion of chromatin material from the egg nucleus in the form of karyosomes has also been demonstrated. It is admitted that in the light of our meager knowledge of cellular function no definite suggestion can be offered at present regarding the functional significance of the extruded chromatin found in the oöplasm in the form either of follicular nuclei or of karyosomes.

A discussion on the functional significance of the follicle would be incomplete without mentioning its fate after ovulation. The cells of a ruptured follicle invade the follicular space and form a new structure, the corpus luteum. The presence of a corpus luteum was noted in both the oviparous and ovoviviparous forms as also reported by other workers. The corpus luteum bears a striking resemblance to similar structures in mammals (Fig. 9), but further research is needed to establish definitely its structural and functional homology to the mammalian corpora lutea. Weekes (1934) found in certain European and Australian lizards a similar structure. He has made a comparative study of the

corpus luteum in these forms to determine its role during the variable periods of retention of eggs or embryos inside the oviduct.

The findings of Clausen (1940) regarding the physiological significance of the corpora lutea on gestation in snakes are of importance. He has shown experimentally by means of ovariotomy and hypophysectomy that the mechanism of gestation and parturition in snakes are controlled by similar factors as in some species of mammals. No attempt to determine the functional role of the corpora lutea was made in this study, but its structural resemblance to the mammalian corpora lutea and the evidence offered by Clausen (1940) indicate that it does play some part in the retention of intra-uterine eggs or embryos.

Plasmosome and Plasmosomal Extrusion

In this investigation I have studied two kinds of nucleolar bodies, the plasmosomes and karyosomes. However, this discussion mainly concerns the plasmosome (true nucleolus) because a large amount of literature has appeared recently pertaining to this body. Among the earlier contributions on the origin, structure, and function of the plasmosome mention may be made of the work of Montgomery (1899), Meyer (1917), Nakahara (1917), Ludford (1922), Gardiner (1927, 1935), Sato (1928), Mottram (1932), Gresson (1929a, 1934), and Johnson (1938). In spite of all this detailed work on the plasmosome our knowledge regarding its structure and functional

significance has remained obscure. Recently it has received attention anew at the hands of skilled investigators, and a new concept of its functional relationships has come into existence through the investigations of Caspersson and Schultz (1940), Brachet (1940a, 1940b, 1947), Schultz, Caspersson and Aquilonius (1940), Painter and Taylor (1942) and Painter (1945).[1]

There are two possible theories regarding the origin of the plasmosome: (1) intranuclear, and (2) extranuclear. All the available evidence is in favor of the former theory, although the actual mode of its origin is a matter of controversy. My work does not deal with the cytogenetical considerations but a few pertinent views have been included as discussed by Schultz, Caspersson, and Aquilonius (1940). According to their views the plasmosome is related to a definite locus in what they term the heterochromatin. These heterochromatin regions have the capacity in Drosophila (1) to form a large amount of thymonucleic acid in the chromosomes themselves, (2) to form or effect the composition of the plasmosome, and (3) to effect the content of ribonucleic acid in the egg cytoplasm. The evidence at hand seems to show that the plasmosome is mainly concerned with the ribonucleic acid metabolism in the cell (Caspersson and Schultz, 1940).

It is well known that the plasmosome is very variable in its form and staining capacity. The changeable nature of the plasmosome has also been emphasized in a recent paper

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[1] These workers do not differentiate nucleoli into plasmosome and karyosome so that in my report "plasmosome" is used synonymously with their "nucleolus."

by Painter (1945). Perhaps these variations are linked with its function, which is concerned with the secretory activities of the cell. It is a significant observation that the plasmosomes are prominent in cells connected with secretion and are inconspicuous in cells undergoing cleavage. Its absence in spermatids lends further support to the view that it has mainly a synthetic role in growing cells. However, variations in staining reactions of the plasmosome may be due to (1) variable concentrations of its constituent substances, (2) differences in physical nature of the substances, and (3) differences produced by fixing and staining methods. Sato (1928) has dealt in detail with this subject of staining capacity of the plasmosome and its physiological significance. It appears that at present no dependable histochemical and staining methods for determining the true chemical nature of the plasmosome are available, and more reliance should be placed on physical methods such as the ultra-violet spectrophotometric method employed by Caspersson and Schultz (1940).

In the snakes, I have observed the budding process of the plasmosome resulting in the formation of several plasmosomal bodies. The actual process of budding has been rarely described. Subramanium and Gopala Aiyar (1935) have mentioned that they failed to see the actual process. My observations have convinced me that the daughter plasmosomal bodies are formed from the principal plasmosome rather than any postulated chromatinic source. Eventually the extrusion

of these daughter plasmosomes into the egg cytoplasm occurs. Although the emission of plasmosomal bodies has been previously described by several workers, they have failed to provide sufficient experimental evidence in support of their views with the exception of Kater (1928). I have demonstrated the extrusion of plasmosomes in a convincing manner and I have also shown that daughter plasmosomes come out into the cytoplasm as discrete bodies and not in fluid state as suggested by Hilton (1931) in Calanus finmarchicus.

What is the significance of the extruded plasmosomes? An answer to this question will be hypothetical in view of our limited knowledge of biosynthesis. The contributions of Caspersson, Brachet and others, however, provide a partial answer to the question. These workers have suggested that there exists a fundamental connection between the accumulation of nucleic acids in the cytoplasm and protein synthesis. In fact, Caspersson has put forth a theory to explain the mechanism of intracellular protein synthesis. He believes that the nucleus is the center of protein synthesis and that euchromatin is concerned with the synthesis of more complex and specific proteins elaborated by the genes, while heterochromatin is concerned with synthesis of simpler histones. These histones accumulate in the plasmosome and then diffuse into the cytoplasm. The histones induce a synthesis of ribonucleic acid, which would be the final agent of cytoplasmic protein synthesis. This theory is in agreement with known cytological and cytochemical facts, but the only missing link

is between the nucleic acids and protein synthesis. It seems that the extrusion of plasmosomal bodies rich in ribonucleic acid provides a means for the exchange of the nucleic acids from the nucleus into the cytoplasm for protein synthesis. If this be true, the extrusion of the daughter plasmosomes is a very significant process because through these plasmosomal bodies protein or protein precursors are discharged into the cytoplasm. My work provides direct evidence to show that there is an exchange of materials between nucleus and the cytoplasm, but the nature and the role of the extruded materials need to be determined.

Golgi Apparatus

Although several comprehensive reviews dealing with the controversial nature of the Golgi apparatus have appeared periodically (Pappenheimer, 1916; Cowdry, 1923; Bowen, 1926, 1927, 1928, and 1929; Nahm, 1933; Kirkman and Severinghaus, 1938; Bourne, 1942; Baker, 1944; Nath, 1944; Hibbard, 1945; Worley, 1946), our knowledge of this cytoplasmic component still remains inadequate. What is the morphology of the Golgi apparatus in the growing oöcyte, and what is its function? The present investigation throws some light on these two important problems. A detailed review of the literature on the Golgi apparatus has not been attempted and only the important conclusions reached by other workers are discussed.

Investigators on the Golgi apparatus can be classified into three groups on the basis of the conclusions they have

reached: (1) those who deny the existence of a Golgi apparatus as a structural unit and regard it as a mere chemical product of physiological processes (Walker and Allen, 1927; Tennent, Gardiner, Smith, 1931), (2) those who homologize it with the chondriome or vacuome (Parat and Painlevé, 1924: Parat, 1928;), and (3) those who think of it as a specific entity.

Most cytologists regard the Golgi apparatus as an independent cytoplasmic structure capable of propagation in the cell. Gatenby (1919) has demonstrated in the eggs of Limnaea stagnalis that it is traceable into the blastomeres. If this be true its genetic continuity from generation to generation is established.

There seems to be no doubt about the existence of Golgi elements in oöcytes as evidenced by their orderly behavior during their cycle of activity. The present study provides further evidence in favor of the view that the Golgi apparatus is a separate entity independent of the neutral-red vacuoles. Supravital staining methods have demonstrated the occurrence of Golgi bodies and neutral-red vacuoles side by side, thereby establishing the separate nature of these cytoplasmic structures. Other workers have similarly described them as distinct entities (Nath and Nangia, 1931). It is concluded, therefore, that in spite of the close association of the Golgi apparatus and the vacuome in early oöcytes, these cytoplasmic bodies are distinct from one another and not identical as claimed by Parat and Painlevé

(1924). The main attack on Parat's vacuome theory has come from Gatenby, Bowen, Hirschler, Voinov, Ludford, Beams, Bhattacharya, and Nath, and at present it has very few supporters.

The main difficulty in working with the Golgi apparatus is the lack of reliable techniques for its demonstration. The silver and osmium methods are not without pitfalls and for this reason several other cytoplasmic bodies which have nothing in common with the Golgi apparatus have been mistaken for it. If the standard silver and osmium methods are not specific, the only other criterion of its identity is its structural form. In my preparations, the granules which were defined as Golgi bodies did not have a morphology which differentiated them from other cytoplasmic structures. It should be noted that a system of canals was observed (Fig. 26) which resembles more closely the conventional concept of the form of the Golgi apparatus. However, in my preparations these canals were not impregnated by either silver or osmium. Due to the lack of adequate differentiating techniques there is hardly any agreement among investigators, in general, regarding its morphological character.

As to form, the Golgi bodies have been described as granular, vesicular, platelet-like, crescentic, in networks or in irregular clumps. In some instances, even in the same species different forms have been reported. Harvey in the eggs of Lumbricus terrestris (1925), Ciona intestinalis (1927), and Carcinus moenas (1929) describes the Golgi bodies, as scaly and dictyosomal. Gatenby (1919) in Limnaea stagnalis has also described the Golgi bodies as curved dictyosomes concentrated in the juxtanuclear archoplasmic area in early oöcytes and as dust-like granules in advanced oöcytes. I have found the Golgi bodies, in a few instances, as irregular clumps or in the form of dictyosomal bodies. These forms were rare in my material and were not observed

in the fresh material. It appears, therefore, that such bodies are formed as a result of over impregnation with silver.

Wheeler (1924) in Pleuronectes limanda, Gardiner (1927) in Limulus polyphemus, and Payne (1932) in insects described the Golgi bodies as small, round granules and spheres. I have found them in snakes to be of granular form also. Nath in Crossopriza lyoni (1928), in Culex (1929), and in Pheretima posthuma (1930) showed the Golgi bodies as of vesicular form with a distinct duplex structure consisting of an osmiophobic or argentophobic center surrounded by an osmiophilic or argentophilic cortex. The chromophilic component (Golgi externum of Hirsch, 1939) is considered as the active part of the Golgi bodies and the chromophobic matter (Golgi internum of Hirsch, 1939; para-Golgi of Salazar, 1942) is regarded as their product. A similar vesicular structure of the Golgi bodies has been reported by Nath and Mohan (1929) in Periplaneta americana and Worley (1944) in Mytilus californianus. I have failed, however, to observe such duplex structure either in fixed or fresh materials.

Bhattacharya and his co-workers have described the Golgi bodies of various sizes and shapes such as in the form of filaments, discs, crescents, spherules, chains of granules, and irregular grains. The polymorphic nature of the Golgi apparatus has also been described by Steopoe(1926) in Nepa cinerea. It is likely that some of these described forms are due to over-impregnation.

In view of such conflicting opinions about the structure of the Golgi body, it seems best as suggested by Bowen to regard it as a substance rather than an individualized organelle. Worley (1946) has given a comprehensive account of the different phases of the Golgi apparatus, namely, the generalized Golgi body, Golgi droplet, Golgi granule, active Golgi body, and pycnotic and multiplication phases of the Golgi bodies. He thinks that the Golgi apparatus in the growing oöcyte is the "manifestation of a series of more or less spherical Golgi bodies of the living cell." Evidently Golgi bodies show a great variability in their total chemical composition which is reflected in marked differences in their structure. Mitochondria, on the contrary, are relatively more stable in form and chemical composition.

In conclusion, it may be stated that a standard form of the Golgi bodies can not be insisted upon. Although the Golgi bodies are capable of existing in other forms, the granular or spherical form seems to be of more general occurrence. A priori one might expect the spherical shape for any body of plastic form suspended in the polyphasic colloidal system which is protoplasm. I am of the opinion, therefore, that the Golgi bodies are basically granular or spherical, but they may show a duplex structure in some cases after undergoing growth. All other forms such as networks, dictyosomes, rods and platelets are perhaps the result of poor fixation.

Fortunately, there is more agreement with regard to the function of the Golgi bodies than as to its structure. Gatenby, Ludford, Brambell, Bhattacharya and Nath have shown that the Golgi apparatus is concerned with the elaboration of fatty yolk bodies during oögenesis. I have reached the same conclusion and have demonstrated direct transformation of the Golgi bodies into the fatty yolk bodies.

Vitellogenesis

Even though the problem of yolk formation has been variously interpreted, there is general agreement with regard to the formation of the fatty yolk. It is considered to be formed under the influence of the Golgi elements (Gatenby and Woodger, 1920; Gatenby, 1922a; Ludford, 1921a; Wheeler, 1924; Brambell, 1924; Gresson, 1929b, 1931; Worley, 1944). These workers, however, do not agree about the actual method of its formation. Some have expressed the view that the Golgi dictyosomes secrete the fatty yolk, while others have shown that there is a direct transformation of the Golgi bodies. My findings are in agreement with the latter view. Nath and Mehta (1929) in Luciola gorhami and Bhandari and Nath (1930) in Dystercus cingulatus have demonstrated the fatty nature of the Golgi elements even in the early oöcytes but I have not observed their presence in early stages. It is concluded, therefore, that fatty yolk formation

is initiated late in the growth of the oöcyte in the snakes which I have studied.

With regard to the formation of proteid yolk, widely divergent views are held among cytologists. In the literature, its source has been traced to the Golgi elements (Harvey, 1925, 1927), mitochondria (Brambell, 1924, 1926; Narain, 1930; Das, 1939), nucleolus (Nath and Mehta, 1929; Nath and Mohan, 1929), and vacuome (Nath and Nangia, 1931). In view of such conflicting opinions, one is inclined to believe that more than one of these cytoplasmic bodies may be concerned in the formation of proteid yolk. The other view, has been expressed by Hibbard (1928), Harvey (1929), and Payne (1932), namely, that the yolk is elaborated independently in the cytoplasm without the intervention of any of these cytoplasmic bodies, which seems to be unwarranted.

Generally the workers on vitellogenesis have not traced the fatty and proteid yolk bodies to a common source. In some instances, however, both kinds of yolk have been traced back to the Golgi elements (Subramaniam and Gopala Aiyar, 1936; Worley, 1944). Also Worley and Worley(1943) have reported that the Golgi bodies discharge both fatty and proteid yolk during the embryonic development of the tectibranch, Navanax. I have failed to find any relationship between the proteid yolk bodies and the Golgi elements.

Gatenby (1922a) in Saccocirrus, Nath and Mehta (1929) in Luciola gorhami, Nath and Mohan (1929) in Periplaneta americana, and Gresson (1929a, 1929b, 1931) in Tenthredinidae

and Periplaneta orientalis have shown the direct transformation of extruded plasmosomal bodies into proteid yolk. Although I have demonstrated the extrusion of plasmosomal bodies in this investigation, I have failed to see any direct conversion of these bodies into proteid yolk. It is plausible, however, that these extruded plasmosomal bodies on dissolution in the cytoplasm provide essential activating chemical substances which do help indirectly in the elaboration of protein bodies. Very little is known at present regarding the biochemical mechanism of protein synthesis and it is not possible to suggest the exact manner in which the extruded plasmosomal bodies may act as agents of protein synthesis. The main difficulty seems to be a lack of biochemical link between the nucleic acids and protein synthesis.

SUMMARY

1. This study, which pertains to three Indian snakes and one American snake, concerns the extensive changes that occur in the oöcyte during oögenesis.

2. The ophidian ovary is saccular and possesses an internal cavity, the ovocoele, into which project the advanced oöcytes. The oöcytes are attached to the ovarian stroma by means of a mesentary through which the oöcytes are supplied with blood vessels.

3. The outer surface of the ovary is composed of a germinal epithelium consisting of two kinds of cells, namely, oögonia and epithelial cells. Early oöcytes may be distinguished by the possession of a prominent spireme within the nucleus during the early prophase stages.

4. The oöcytes in early stages of differentiation are without a follicle. Later, the presumptive follicle cells of the germinal epithelium arrange themselves around the oöcyte to form a single-layered primary follicle, which soon becomes double layered. A fully differentiated follicle consists of (1) a zona radiata with distinct radial striation, (2) three layers of follicle cells and (3) a theca folliculi. The cells of the middle layer of the follicular epithelium are large, spindle-shaped and, apparently, secretory in function. The cells of both inner and outer layers are small and rounded in appearance. The follicle is a

highly developed and differentiated structure in snakes and plays an important part in the growth of the oöcyte.

5. After ovulation the follicle cells invade the space left by the oöcyte and form a new structure, the corpus luteum. This structure was present in both oviparous and ovoviviparous snakes, but its functional significance was not determined.

6. Two kinds of nucleoli are present in the oöcyte: plasmosomes and karyosomes. The plasmosome in the oögonia and in very young oöcytes occurs as a single, rounded body within the nucleus. It is non-chromatinic and negative to the Feulgen reaction. On the other hand, the karyosome is chromatinic in nature.

7. Terms such as "basophilic" or "acidophilic" cannot suitably be applied because the plasmosome is stainable sometimes with acidic dyes and at other times with basic dyes. This suggests that the staining nature of the plasmosomes is partly determined at the time of fixation depending on whether it has absorbed the acidic portion or the basic portion from the fixative mixture. If a basic stain is employed, the affinity of the plasmosome for it will be determined by the content of ribonucleic acid. These changes in the chromaticity of the plasmosomes may also be due to variations in the concentration of nucleic acids present inside of the plasmosomes. Perhaps a variety of physical and chemical factors are responsible for this variation in the stainability of the plasmosomes.

8. The plasmosome grows with the growth of the oöcyte and on reaching a certain size it begins to fragment. Several smaller bodies are formed which are generally of about the same size. These daughter plasmosomal bodies arrange themselves near the nuclear membrane.

9. Extrusion of both plasmosome and karyosome fragments was demonstrated. However, elimination does not continue during the whole period of growth. In the light of recent work of Caspersson and Schultz (1940), the extruded plasmosomes are, perhaps, a means of supplying ribose nucleic acid and other essential materials for protein synthesis in the growing oöcyte. The plasmosome should not be regarded as an ergastic body nor should it be considered as an aggregation of waste materials. It is a body which undergoes specific growth changes during oögenesis and delivers essential materials into the oöplasm for biosynthesis. The functional significance of the extruded karyosomes is problematical.

10. In Lycodon aulicus aulicus, which provides excellent material for the study of nuclear and nucleolar behavior, the nuclei of follicular cells are seen to migrate into the oöplasm. Chromatin is, therefore, emitted into the oöplasm from two sources: from the nucleus of the oöcyte and through the nuclei of follicular cells. It is not possible at present to suggest the exact role played by this extruded chromatin material from the two sources.

11. Golgi bodies appear granular or spherical as seen in fixed and fresh preparations treated with osmic acid.

12. The Golgi apparatus occupies a juxtanuclear position in young oöcytes. This area is the center of cytoplasmic activity and is the focal point for growth and dispersal of the Golgi material. The Golgi apparatus grows with the growth of the oöcyte and soon assumes a perinuclear position. Later, the Golgi bodies gradually disperse in the oöplasm and become evenly scattered and arrange themselves principally in the peripheral region of the oöcyte.

13. Infiltration of the follicular Golgi bodies into the oöplasm has also been observed. A thick peripheral concentration of the Golgi elements comprising the follicular Golgi bodies and the Golgi bodies derived from the oöcyte proper is thus formed. A free intermingling of the Golgi bodies from the two sources occurs.

14. The Golgi granules aggregate to form fatty yolk by direct transformation. Fatty yolk bodies in contrast to the Golgi elements are soluble in fat solvents. They are larger than the Golgi granules and are blackened more quickly with osmic acid.

15. Formation of fatty yolk takes place in the peripheral region of the oöcyte in Eryx conicus and Thamnophis ordinatus ordinatus. It begins at one end of the oöcyte in Natrix piscator piscator but in Lycodon aulicus aulicus it takes place in the "yolk nucleus area."

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Explanation of Figures

Fig. 1 A section showing the germinal epithelium with several oöcytes and the ovarian stroma. (Lycodon aulicus aulicus). Bouin, Mann's methyl blue eosin. x 280.

Fig. 2 A portion of the germinal epithelium showing the prominent, coiled spireme present during the early prophase stages of the first maturation division. A few epithelial cells constituting an early stage in the formation of a follicle are seen around the large oöcyte. (Lycodon aulicus aulicus). Bouin, Mann's methyl blue eosin. x 630.

1

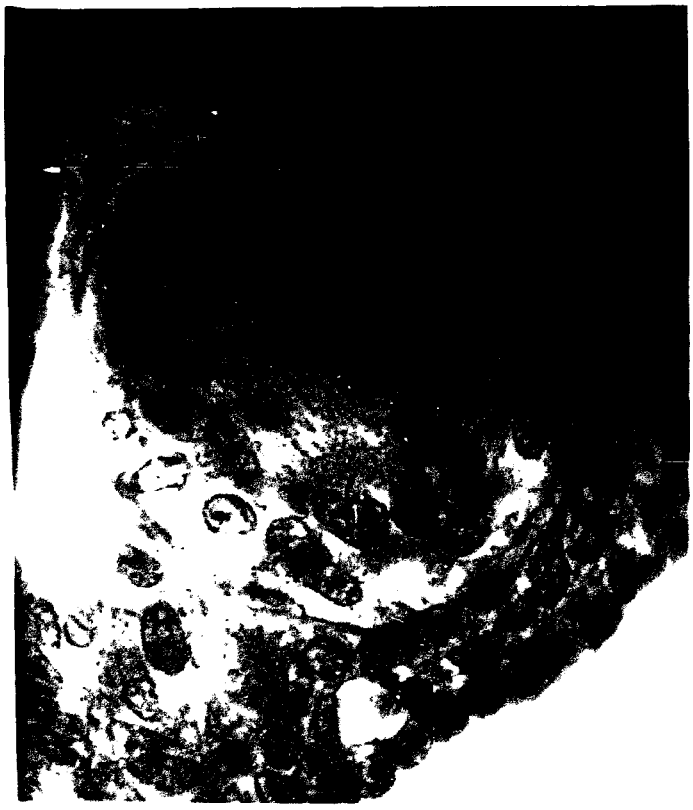
2

Explanation of Figures

Fig. 3 A portion of the germinal epithelium showing presumptive follicular epithelial cells and several oöcytes in the prophase stages of the first maturation division. Note large binucleate oöcyte in the center of the section. (Natrix piscator piscator). Mann's methyl blue eosin. x 630.

Fig. 4 An early oöcyte showing a stage in the formation of the follicle. The epithelial cells at this stage are arranging themselves around the oöcyte to form the primary follicle. (Lycodon aulicus aulicus). Bouin, Mann's methyl blue eosin. x 630.

3



4

Explanation of Figures

Fig. 5 A young oöcyte showing the orderly arrangement of the epithelial cells to form a double layered primary follicle. Notice the absence of follicles around the two adjacent oöcytes. (Lycodon aulicus aulicus). Bouin, Mann's methyl blue eosin. x 630.

Fig. 6 An oöcyte, with a nucleus and plasmosome, showing further differentiation in the development of the follicle. At this stage the oöcyte begins to protrude into the ovocoele. At one end of the follicle are shown three oöcytes which failed to develop. (Thamnophis ordinatus ordinatus). Bouin, Mann's methyl blue eosin. x 140.

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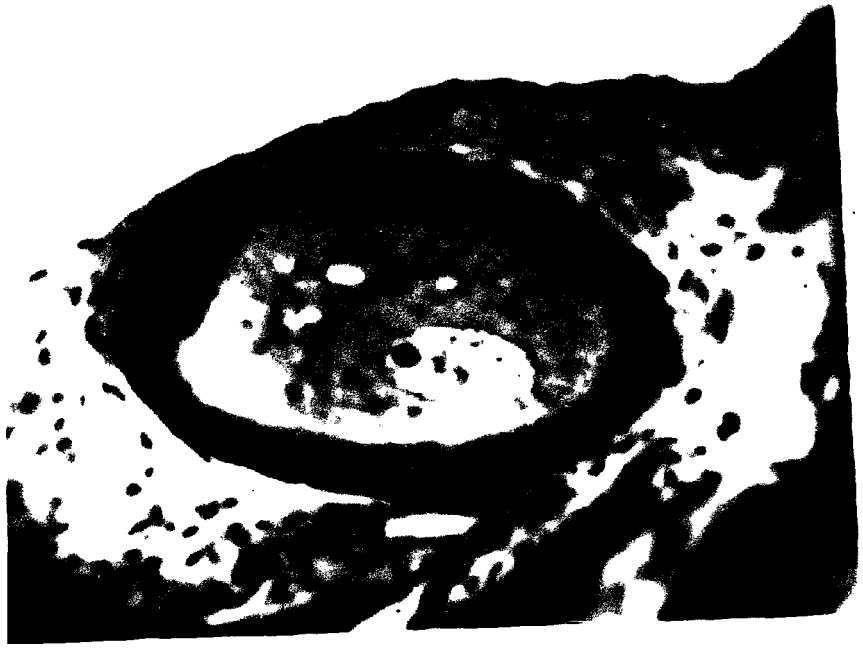

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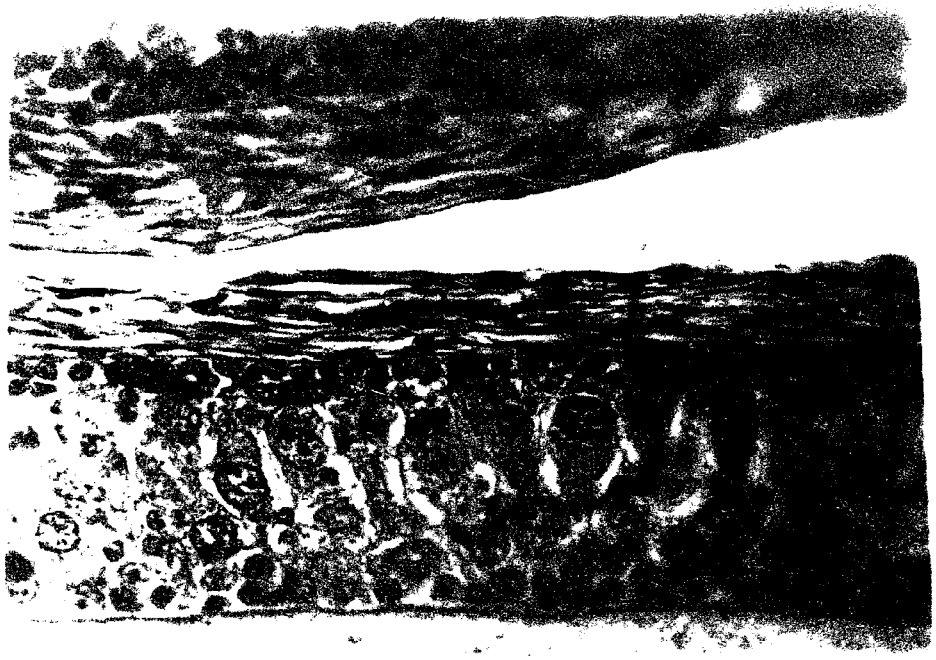
Explanation of Figures

Fig. 7 A part of the follicle of an advanced oöcyte showing the structure of the fully differentiated follicle. (Lycodon aulicus aulicus). Bouin, Mann's methyl blue eosin. x 280.

Fig. 8. A section showing one of the large blood vessels which supply nourishment to the advanced oöcyte. (Thamnophis ordinatus ordinatus). Bouin, Mann's methyl blue eosin. x 70.



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Explanation of figures

Fig. 7 A portion of an advanced oöcyte showing the structure of the fully differentiated follicle. (Lycodon aulicus aulicus). Bouin, Mann's methyl blue eosin. x 280.

Fig. 8. A section showing one of the large blood vessels which supply nourishment to the advanced oöcytes (Thamnophis ordinatus ordinatus [illegible], Mann's methyl blue eosin.

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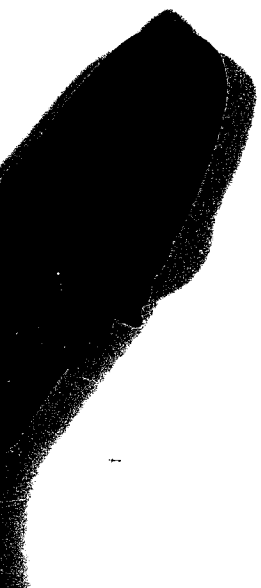
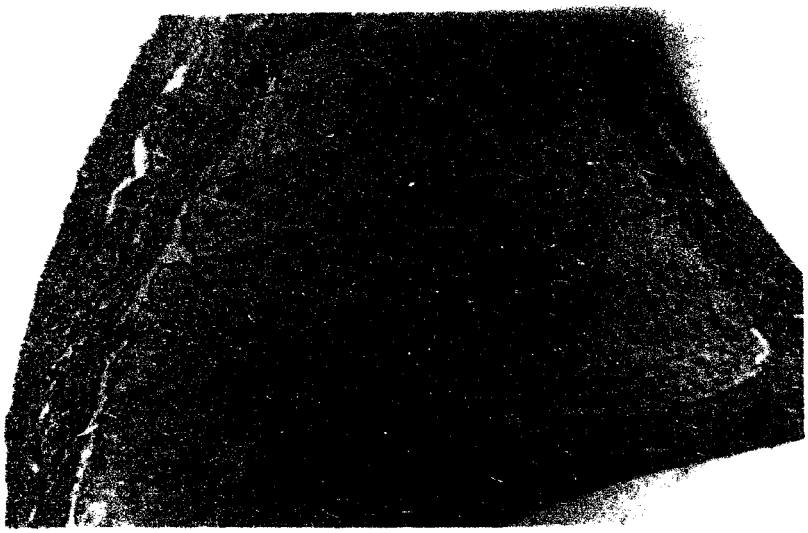
Explanation of Figures

Fig. 9 A portion of the ovary in a mature female showing the corpus luteum formed after ovulation. (Thamnophis ordinatus ordinatus). Bouin, Iron alum hematoxylin. x 150.

Fig. 10 Nucleus of an advanced oöcyte showing the position of the plasmosome near the nuclear membrane. (Thamnophis ordinatus ordinatus). Bouin, Harris alum hematoxylin. x 280.





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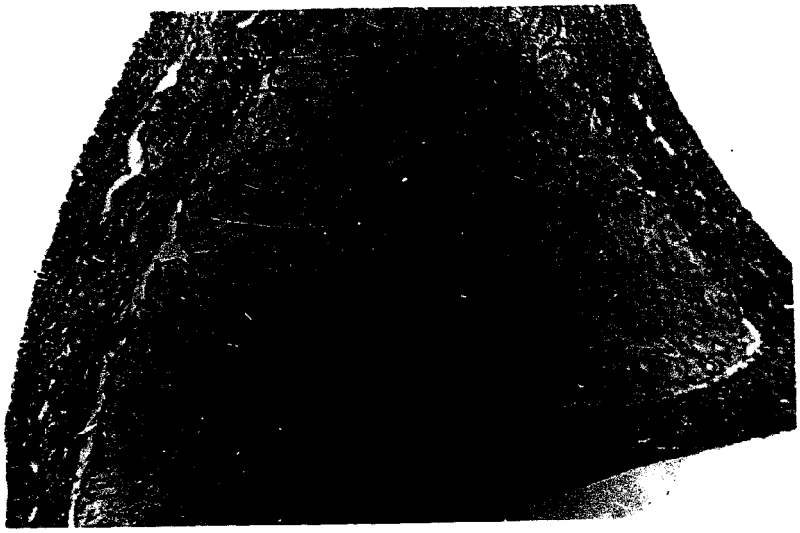
[illegible] ovary in a mature female
[illegible]rous luteum formed after ovula-
[illegible] Thamnophis ordinatus ordinatus).
Bouin, Iron alum [illegible]oxylin. x 150.

Fig. 10 Nucleus of an advanced oöcyte showing the position of the plasmosome near the nuclear membrane. (Thamnophis [illegible]atus ordinatus).
Bouin, Harris [illegible]in. x 280.



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Explanation of Figures

Fig. 11 Nucleus of an advanced oöcyte showing budding of the plasmosome to form daughter plasmosomal bodies. (Thamnophis ordinatus ordinatus). Bouin, Harris alum hematoxylin. x 280.

Fig. 12 Plasmosome in the process of budding inside the nucleus of an advanced oöcyte. Notice the several plasmosomal bodies formed in the process. (Thamnophis ordinatus ordinatus). Bouin, methylene blue eosin. x 630.

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Fig. 13 An advanced oöcyte showing extrusion of the plasmosomal bodies from the nucleus into the cytoplasm. (Natrix piscator piscator). Bouin, Mann's methyl blue eosin. x 140.

Fig. 14 Section showing plasmosomal bodies inside the nucleus and cytoplasm of an oöcyte. One body is lying half within the nucleus and half within the cytoplasm. (Natrix piscator piscator). Bouin, Mann's methyl blue eosin. x 280.

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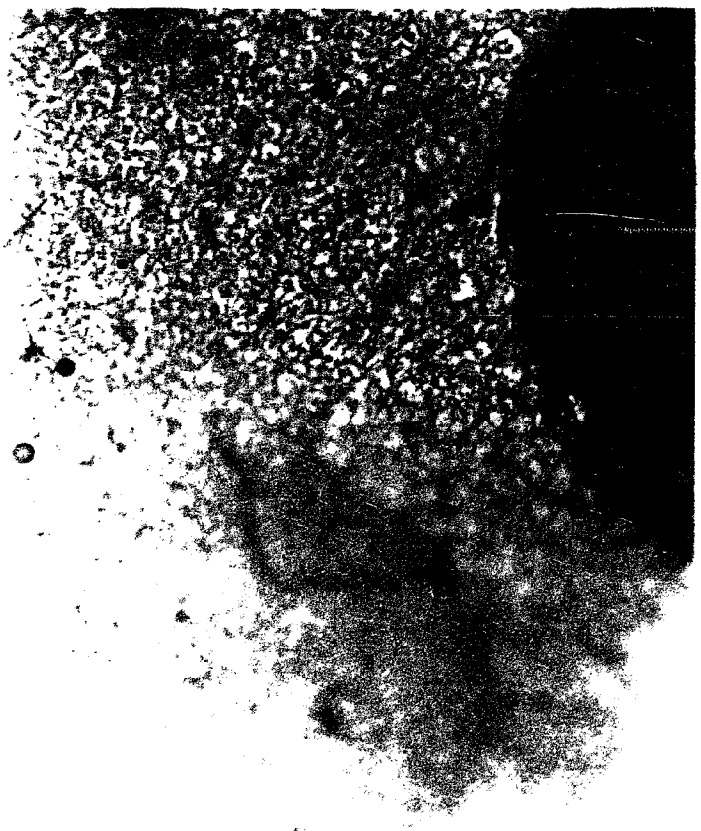

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Fig. 14 Section showing plasmosomal bodies inside the nucleus and cytoplasm of an oöcyte. One body is lying half within the nucleus and half within the cytoplasm. (Natrix piscator). Bouin, Mann's methyl blue eosin. x 140.

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Fig. 15 Section of oöcyte showing spaces inside the nucleus in which are located the plasmosomal bodies before they are extruded into the cytoplasm. Notice an extruded daughter plasmosome. (Eryx conicus). Bouin, Mann's methyl blue eosin. x 140.

Fig. 16 A part of an oöcyte showing (a) several small extruded plasmosomes and (b) a single larger karyosome. (Lycodon aulicus aulicus). Bouin, methylene blue eosin. x 150.

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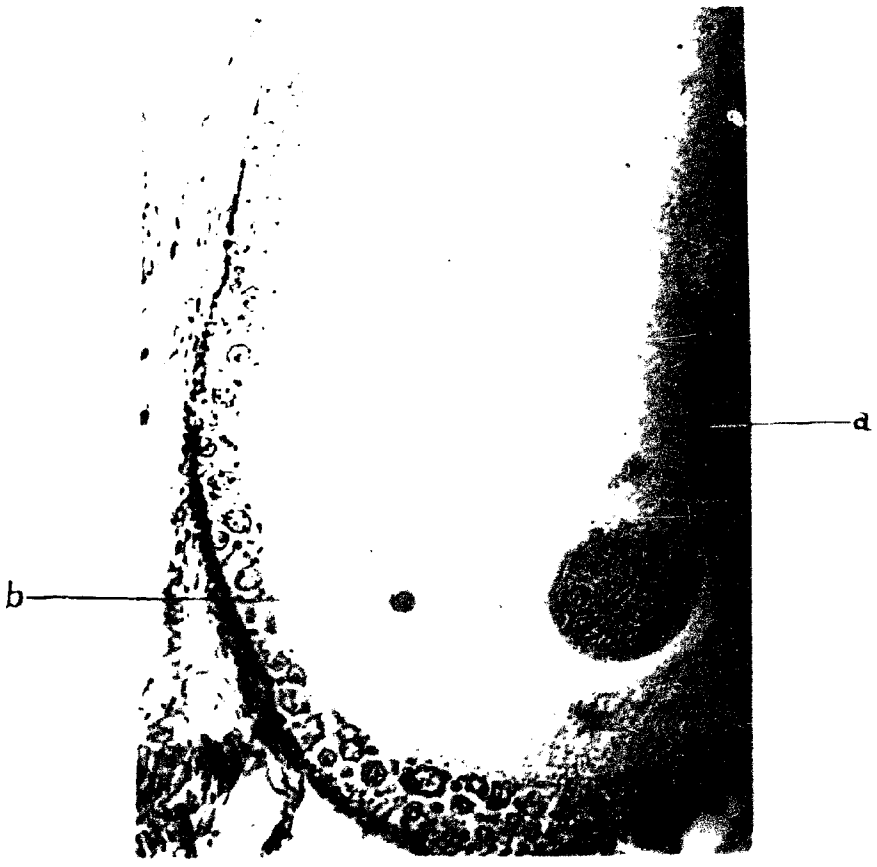

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Explanation of Figures

Fig. 15 Section of oöcyte showing spaces inside the nucleus in which are located the plasmosomal bodies before they are extruded into the cytoplasm. Notice an extruded daughter plasmosome. (Eryx conicus). Bouin, Mann's methyl blue eosin. x 1[illegible].

Fig. 16 A part of an oöcyte showing (a) several small extruded plasmosomes and (b) a single larger karyosome. (Lycodon aulicus aulicus). Bouin, methylene blue eosin. x 150.

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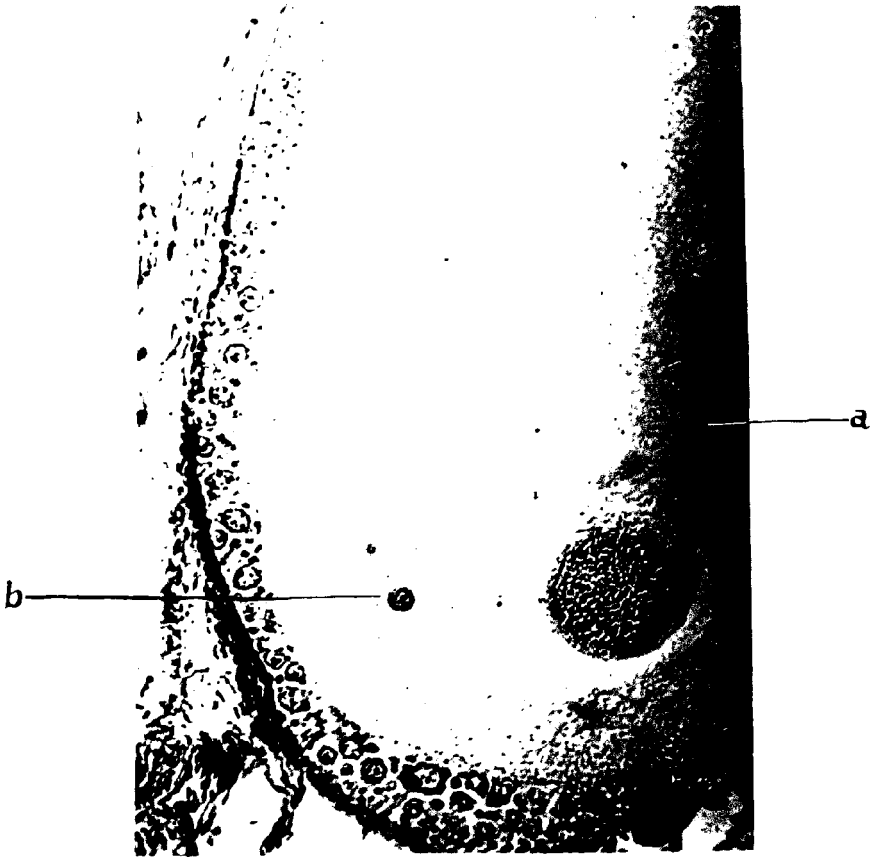

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Explanation of Figures

Fig. 17 A portion of the nucleus of a young oöcyte showing the karyosome lying in a space. (Lycodon aulicus aulicus). Bouin, methylene blue eosin. x 1425.

Fig. 18 A portion of the young oöcyte showing two karyosomes. One of them is in the process of coming out in the cytoplasm and is seen apparently pushing against the nuclear membrane. (Lycodon aulicus aulicus). Bouin, methylene blue eosin. x 900.

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Explanation of Figures

Fig. 17 A portion of the nucleus of a young oöcyte showing the karyosome lying in a space. (Lycodon aulicus aulicus). Bouin, methylene blue eosin. x 1425.

Fig. 18 A portion of the young oöcyte showing two karyosomes. One of them is in the process of coming out in the cytoplasm and is seen apparently pushing against the nuclear membrane. (Lycodon aulicus aulicus). Bouin, methylene blue eosin. x 900.

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Fig. 19 A portion of the cytoplasm in an advanced oöcyte showing an extruded karyosome. Same karyosome as shown in figure 16. (Lycodon aulicus aulicus). Bouin, methylene blue eosin. x 1425.

Fig. 20 A portion of an advanced oöcyte showing the migration of two follicular nuclei into the oöplasm. (Lycodon aulicus aulicus). Bouin, Mann's methyl blue eosin. x 900.

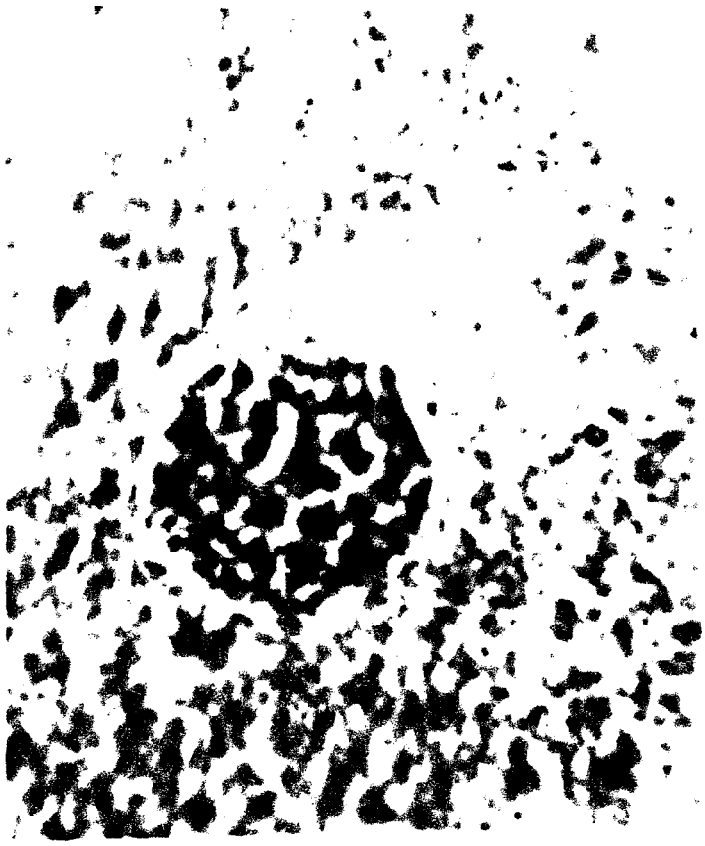

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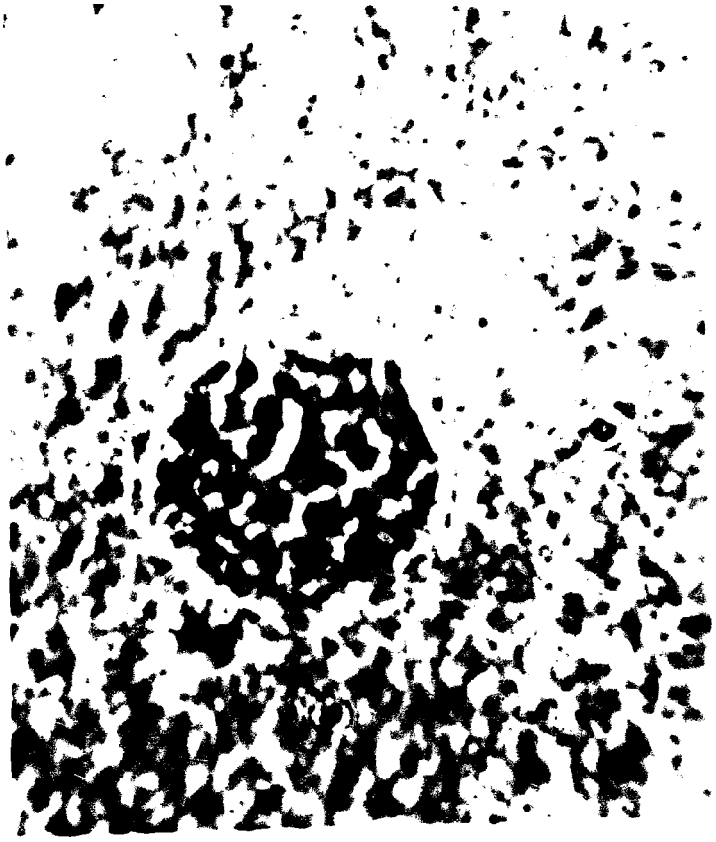
Fig. 19 A portion of the cytoplasm in an advanced oöcyte showing an extruded karyosome. Same karyosome as shown in figure 16. (Lycodon aulicus aulicus). Bouin, methylene blue - eosin. x 1425.

Fig. 20 A portion of an advanced oöcyte showing the migration of two follicular nuclei into the cytoplasm. (Lycodon aulicus aulicus). Bouin, Mann's methyl blue eosin. x 900.



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Explanation of Figures

Fig. 21 A portion of the germinal epithelium showing juxtanuclear mass of the Golgi granules in an early oöcyte. (Natrix piscator piscator). Ludford, bleached. x 950.

Fig. 22 A portion of the germinal epithelium showing juxtanuclear concentration of the Golgi bodies in an early oöcyte. (Eryx conicus). Ludford, bleached. x 950.

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germinal epithelium showing mass of the Golgi granules in an oöcyte. (Natrix piscator piscator). Ludford, bleached. x 950.

Fig. 22 A portion of the germinal epithelium showing juxtanuclear concentration of the Golgi bodies in an early oöcyte. (Eryx conicus). Ludford, bleached. x 9[illegible].

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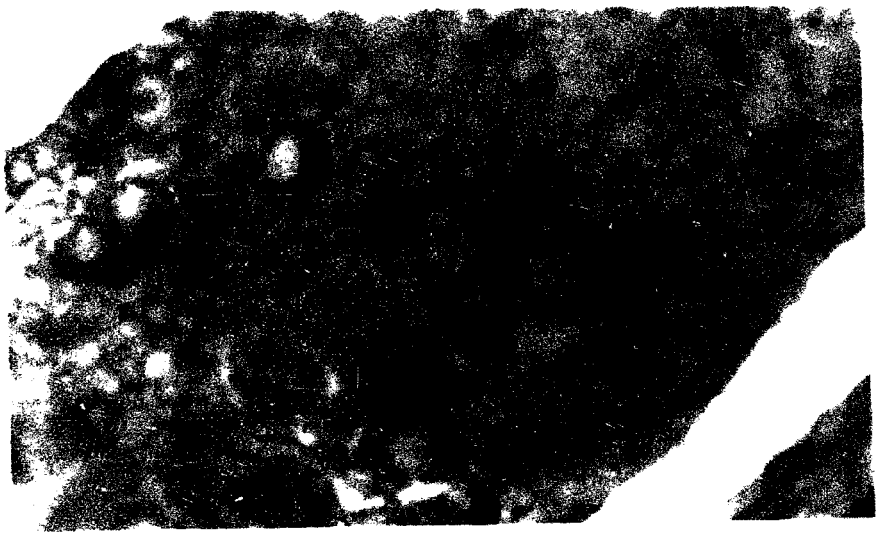
Fig. 23 A portion of the germinal epithelium showing perinuclear concentration of Golgi bodies in the oöcyte (Natrix piscator piscator). Ludford, bleached. x 630.

Fig. 24 Young oöcyte showing the growth of the Golgi apparatus. The original juxtanuclear position is still apparent. (Natrix piscator piscator). Ludford, bleached. x 150.



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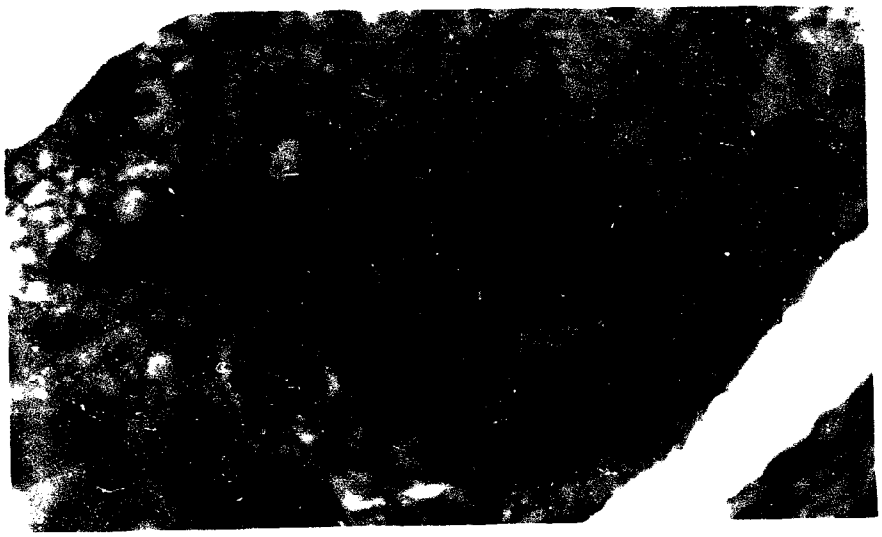
Explanation of Figures

A portion of the germinal epithelium showing perinuclear concentration of Golgi bodies in the oöcyte (Natrix piscator piscator). Ludford, bleached. x 630.

Fig. 24 Young oöcyte showing the growth of the Golgi apparatus. The original juxtanuclear position is still apparent. (Natrix piscator piscator). Ludford, bleached. x 150.



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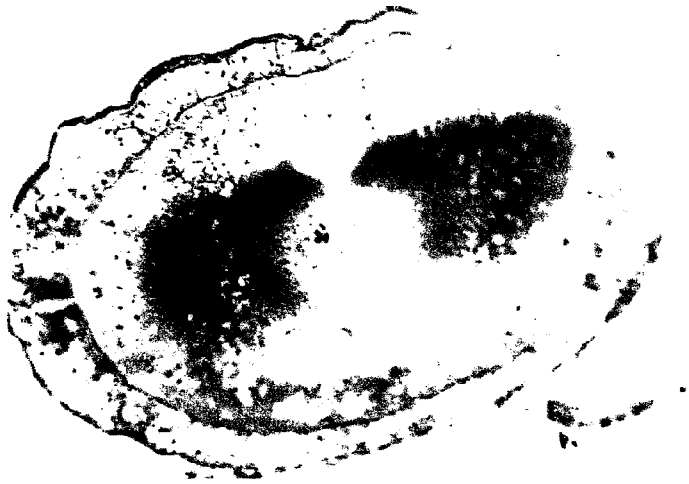
Explanation of Figures

Fig. 25 An oöcyte showing the dispersal of the Golgi apparatus from the "yolk nucleus area" toward the peripheral region of the oöcyte. (Natrix piscator piscator). Ludford, bleached. x 150.

Fig. 26 A part of the oöcyte and its follicle showing the peripheral concentration of the Golgi bodies after the dispersal stage. (Natrix piscator piscator). Cajal, toned. x 100.



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(Natrix piscator piscator). Cajal, toned.
x 100.

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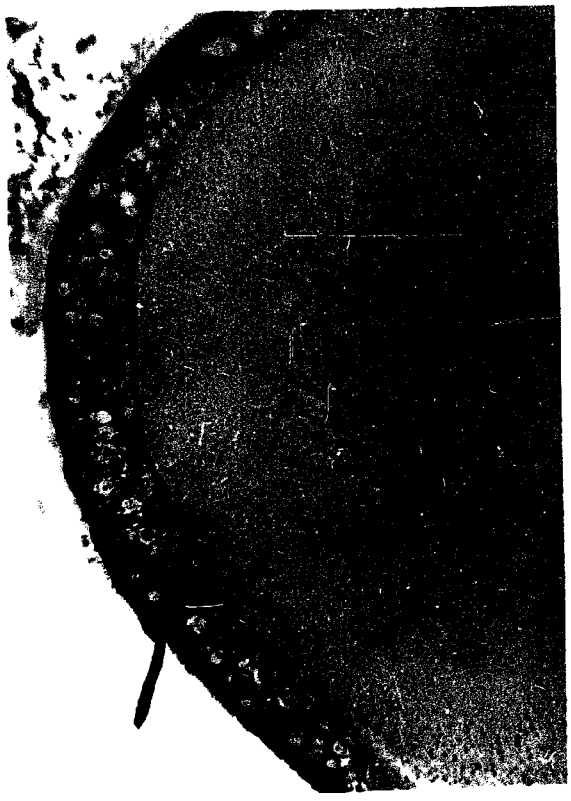

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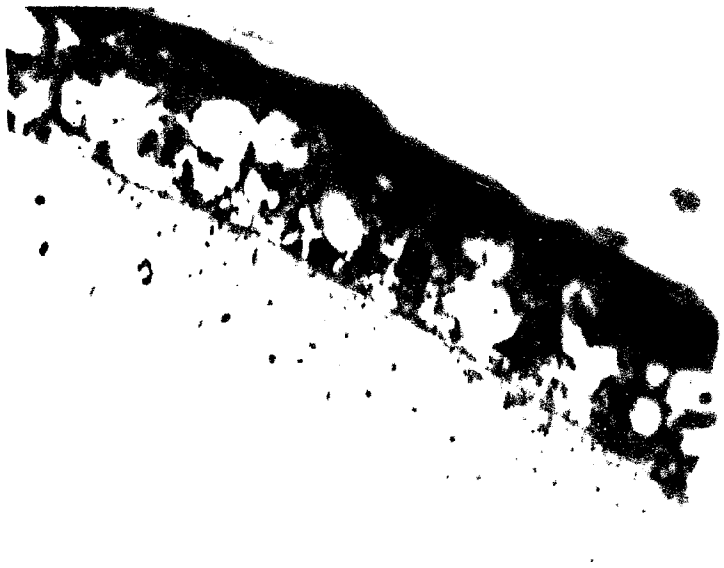
Explanation of Figures

Fig. 27 A part of an advanced oöcyte and its follicle showing the peripheral arrangement of the Golgi bodies in the oöcyte. Note the Golgi bodies of the follicular epithelium. (Lycodon aulicus aulicus). Da Fano, toned. x 600.

Fig. 28 A part of the follicle of an advanced oöcyte, showing the Golgi bodies in the follicular epithelium. (Natrix piscator piscator). Aoyama, toned. x 900.



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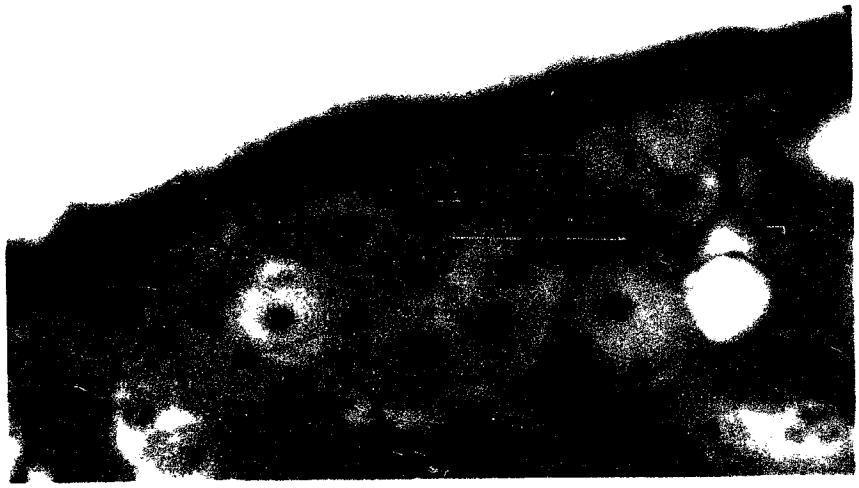

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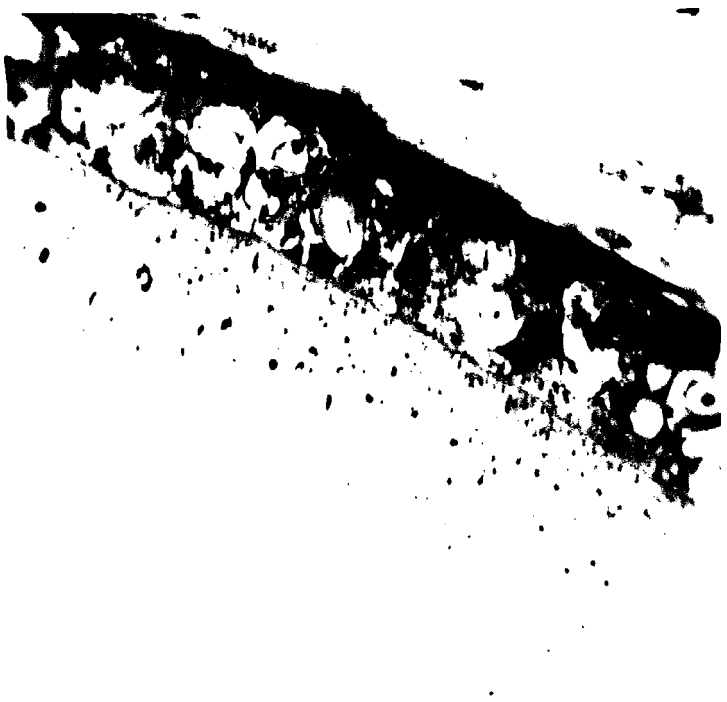
Fig. 27 A part of [illegible] and its follicle [illegible] arrangement [illegible] cyte. Note [illegible] licular epithelium). Da Fano,

Fig. [illegible] A part of the follicle of an advanced oöcyte, showing the Golgi bodies in the follicular epithelium. (Natrix piscator piscator). Aoyama, toned. x 900.



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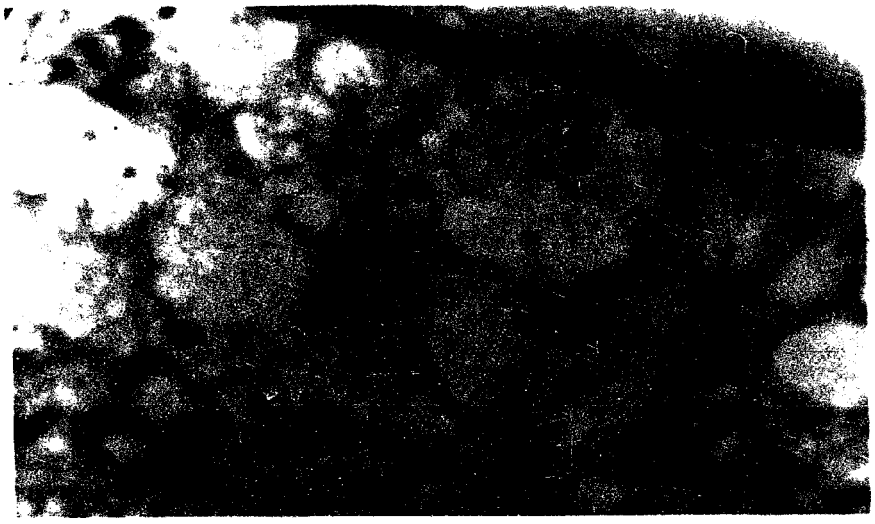
Explanation of Figures

Fig. 29 A part of the follicle in an advanced oöcyte, showing Golgi bodies of various sizes and stages. (Lycodon aulicus aulicus). Aoyama, toned. x 900.

Fig. 30 A part of an advanced oöcyte and its follicle, showing the infiltration of the follicular Golgi bodies through the zona radiata into the oöplasm. (Natrix piscator piscator). Ludford, bleached. x 900.



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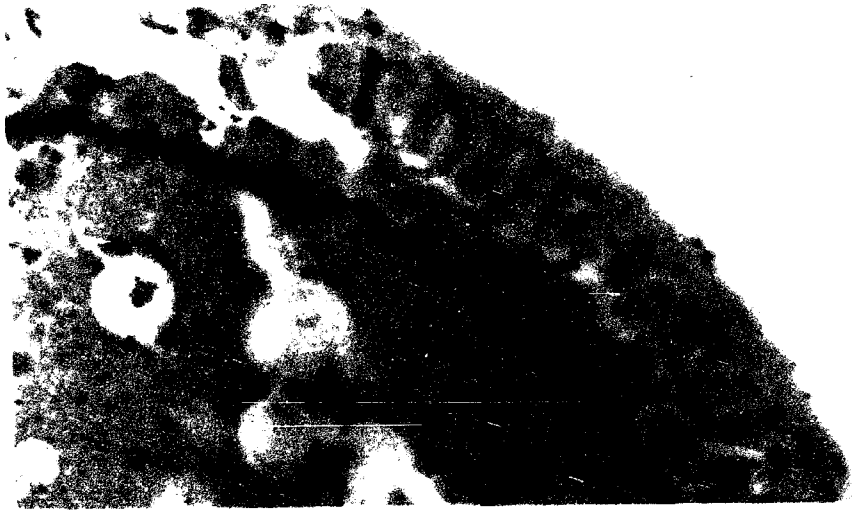

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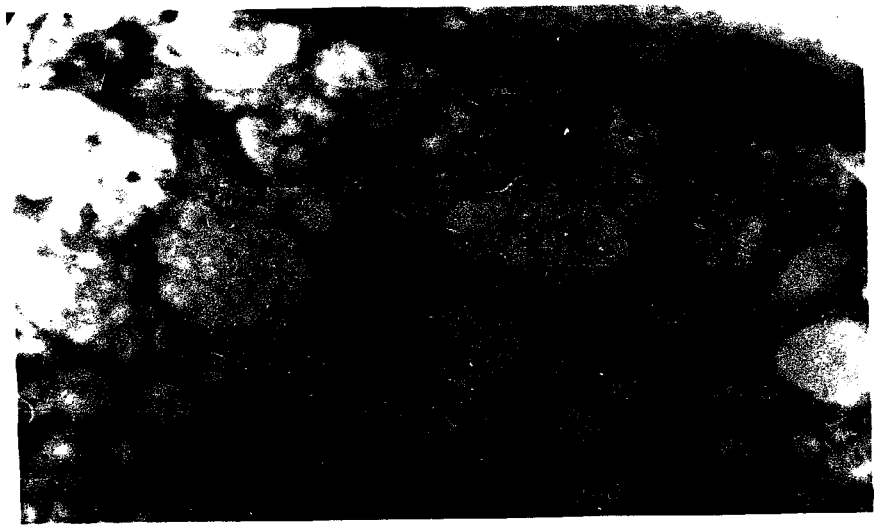
Explanation of Figures

[illegible] part of the follicle in an advanced [illegible] showing Golgi bodies of various [illegible] and stages. (Lycodon aulicus auli- [illegible] A vom', toned. x 900.

[illegible] advanced oöcyte and its fol- [illegible] with the infiltration of the [illegible] ar Golgi bodies through the zona [illegible] olata into the oöplasm. (Natrix pisca- tor piscator). Sudford, bleached. x 900.



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Explanation of Figures

Fig. 31 A part of an advanced oöcyte and follicle, showing that infiltration of the Golgi bodies from the follicle into the oöplasm has been completed. Note the peripheral arrangement of the Golgi bodies in the oöcyte. (Eryx conicus). Da Fano, toned. x 900.

Fig. 32 A part of an advanced oöcyte and follicle, showing a heavy peripheral concentration of the Golgi bodies in the oöplasm, representing a stage shortly before redispersal for yolk formation. (Natrix piscator piscator). Cajal, toned. x 900.

Explanation of Figures

Fig. [illegible] A part of an advanced [illegible]cyte and follicle, showing [illegible] of the Golgi bodies from [illegible] the [illegible]plasm has been [illegible] peripheral arrangement of the [illegible] bodies in the [illegible]te. (*Eryx conicus*). Da Fano, toned. [illegible].

Fig. 32 A part of an advanced [illegible]cyte and follicle, showing a heavy peripheral concentration of the [illegible] bodies in the [illegible], representing a stage shortly [illegible] redispersal for yolk formation. ([illegible] *piscator piscator*). Cajal, toned. [illegible].

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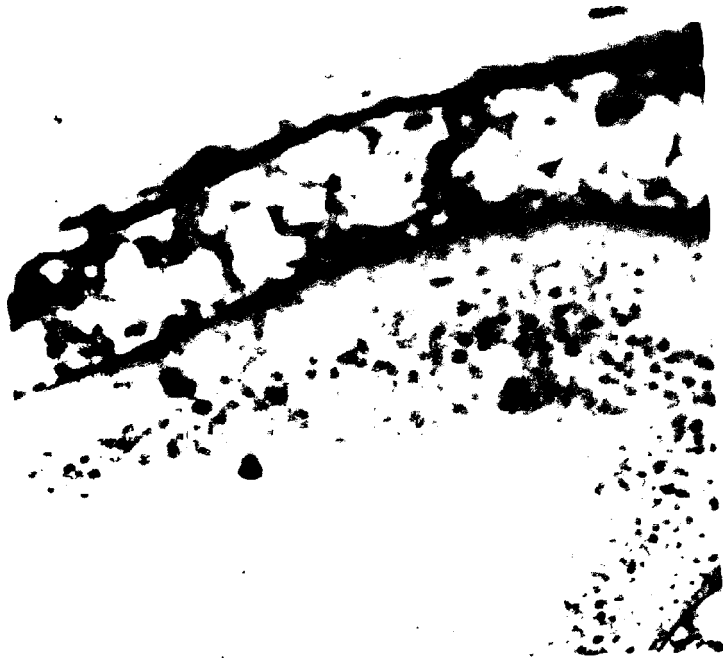

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Explanation of Figures

Fig. 33 A part of the oöcyte showing formation of fatty yolk by direct transformation of the Golgi bodies. (Thamnophis ordinatus ordinatus). Ludford, bleached. x 900.

Fig. 34 A part of an oöcyte showing aggregation of smaller Golgi bodies into larger bodies which are in the process of direct transformation into fatty yolk. (Eryx conicus). Ludford, bleached. x 630.

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A part of an oocyte showing formation of fatty yolk by direct transformation of Golgi bodies. (Thamnophis ordinatus ordinatus). Ludford, bleached. x 900.

Fig. 34 A part of an oocyte showing aggregation of smaller Golgi bodies into larger bodies which are in the process of direct transformation into fatty yolk. (Eryx conicus). Ludford, bleached. x 930.

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Fig. 35 A part of an advanced oöcyte showing an inner concentration of Golgi bodies and fatty yolk formation in the peripheral region of the oöcyte. (Thamnophis ordinatus ordinatus). Ludford bleached. x 150.

Fig. 36 An advanced oöcyte showing the formation of the fatty yolk at one end. (Natrix piscator piscator). Ludford, bleached. x 150.

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Fig. [illegible] an advanced oöcyte showing an [illegible] of Golgi bodies and [illegible] in the peripheral [illegible] cyte. (Thamnophis ordina- [illegible]. Ludford bleached. x 150.

[illegible] An advanced oöcyte showing the formation [illegible] fatty yolk at [illegible]d. (Natrix [illegible]ator piscator [illegible]rd, bleached. x 150.

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Explanation of Figures

Fig. 37 An advanced oöcyte showing formation of fatty yolk in the "yolk nucleus area." (*Lycodon aulicus aulicus*). Ludford, bleached. x 100.

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