OOGENESIS IN THE NILE BOLTI, *TILAPIA NILOTICA* L.

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I.—INTRODUCTION

Since the end of the last century, the study of oogenesis in fishes has attracted the attention of many authors. They are mainly interested in the eludication of the morphological changes which take place in the course of the growth of the oocytes. The whole course of development of the oocytes is divided into stages, phases or periods. Thus, Yamamoto (1956 a), divided the process of differentiation of the oocyte into eleven stages while Kazansky (1949 and Lapitsky (1949) have distinguished four periods in the process of oogenesis in fishes.

In the oocytes of bony fishes and during vitellogenesis are accumulated inclusions of different chemical nature. Scharff (1887), Singh and Boyle (1940) and others related the "yolk nucleus" to the formation of yolk and hence the name. Still others (e.g. Calderwood 1892 ; Subramaniam and Aijar, 1935, etc...) relate yolk nucleus with fat formation. The yolk nucleus is made up of the Golgi apparatus and mitochondria (Narain, 1937, Chaudhry 1952, Mendoza, 1954) and sometimes the centrosome as well (Narian, Chaudhry). Narian (1937) denying the participation of the nucleoli in the formation of food materials, connected the formation of albuminous yolk with mitochondria while fatty yolk related to the Golgi apparatus. A similar relation to the Golgi apparatus was again maintained by Wheeler (1924) Nath and Nangia (1931) and others.

The present work is undertaken in an endeavour for describing the growth phases of the egg of the cichlid, *Tilapia nilotica.*

II.—MATERIAL AND METHODS

Ovaries of the Nile Bolti, *Tilapia nilotica* Linn. were collected in the different periods of the year and fixed immediately in aqueous Bouin or 10% formalin. Paraffin sectioning was undertaken after dehydration and clearing. Sections ranging from 6 to 15 u in thickness were stained in Heidenhain iron haematoxylin or in Heidenhain Azan stain.

III.—OBSERVATIONS

1. *Development of new generations of eggs :*

Microscopic examination of the ovary all the year around, revealed the continuous presence of oogonia and young oocytes. These lie in nests composed of few number of cells. On the whole these nests are not so common and this may be due to the fact that in *T. nilotica* the number of eggs inlined for spawning are much fewer than that of the species laying pelagic eggs.

In the sexually mature fishes, we succeeded to detect oogonial mitotic division only during September (Fig. 1). This dividing cell is usually small, about 10 u in diameter, and shows little affinity to stains. In the metaphase stage of the mitotic division, the chromosomes are packed together, stain deep black by Heidenhian's iron haematoxylin and are surrounded by a thin layer of cytoplasm.

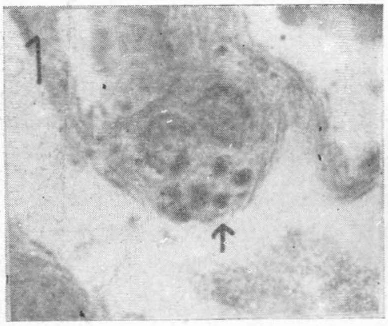

FIG. 1.—Mitotic division of oogonia, ovary fixed on 24/9/1965 (360 X)

Besides, by careful examination of the wall and stroma of the ovary we were unable to detect any figures expressing the possibility of transformation of the peritoneal or connective tissue cells into reproductive cells. This again contradicts Ihring (1883), Calderwood (1892), Ivanov (1951) and others.

Therefore, it appears that the increase in the number of ooganía in the sexually mature fishes occurs through mitotic division. Besides, there are some indications which may reveal that the division of the oogonia takes place. It was possible to identify some, though rare, faintly basophilic oocytes with 2 nuclei (Fig. 2). These oocytes are nearly oval, about 130 and 100 u. along the long and short axes respectively. The nucleus is spherical, about 30 u. in diameter and has a ring of small spherical nucleoli which lie mostly adherent to the nuclear wall.

2. *Period of Synapsis :*

The small oocytes, which are formed by mitotic division pass towards the period of synapsis. These oocytes are minute, about 12 u. in diamter. The nucleus is spherical 10 u. in diameter, occupies almost the whole bulk of the cell and is thus surrounded by a thin layer of cytoplasm. The nucleus has a net-work of chromatin material and comparatively large nucleolus about 2 u. in diameter. The nucleolus in most cases lies or near the central regions of the nucleus (Fig. 3). This oocyte stage is comparable to the presynapsis stage of Marechal (1907). In the synapsis stage, the oocyte undergoes some increase in size. The chromosomes by this stage are highly compact together forming a dense darkly staining mass and at this stage absorption of the nucleoli (Fig. 4) takes place.

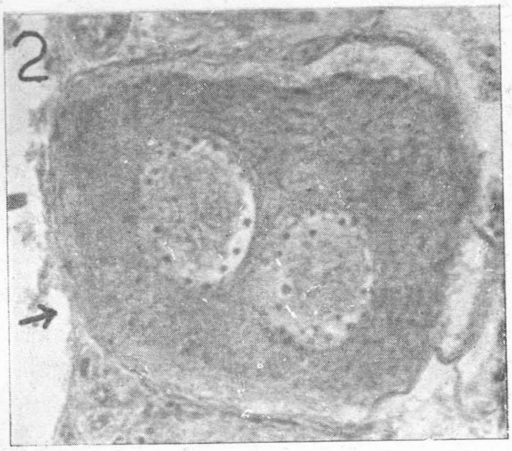

FIG. 2.—Oocyte with two nucleoli, ovary fixed on 24/9/1965 (270 X).

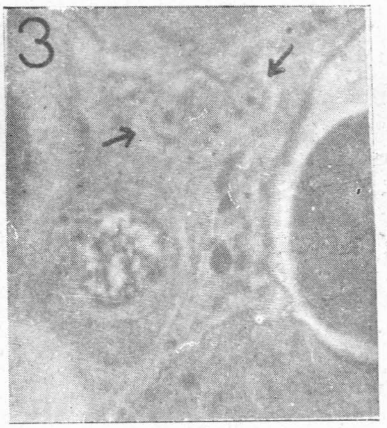

FIG. 3.—Pre-synapsis stage of oocytes (270 X).

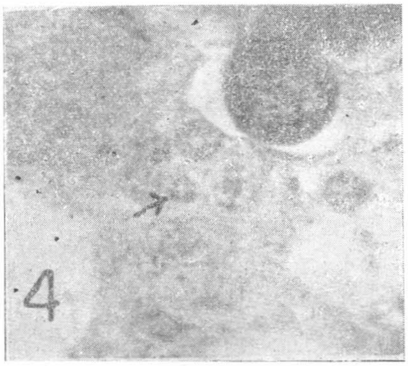

FIG. 4.—Oocyte of synapsis stage (360 X).

Oocytes of the post-synapsis stage are comparatively larger than those of the preceding stage, due to the increase in mass of nucleus and cytoplasm. Oocytes of postsynapsis stage are about 30 u. in diameter. The cytoplasm is in the fixed condition, finely granulated and very faintly basophilic. The nucleus is spherical or slightly oval, and have few small spherical mucleoli close to the nuclear membrane. The chromosomes are in the form of fine coarse threads scattered within the nuclear fluid (Fig. 5).

FIG. 5.—Oocyte of post-synapsis stage (270 X).

3. *Period of protoplasmic growth :*

The characteristic feature of this period is the increase in the size of the oocyte, due to the increase of the mass of the nucleus and the cytoplasm. With such an increase some changes take place in the affinity of the oocytes to stains, distribution of the nucleoli, and the egg-wall.

The early oocytes of the period of protoplasmic growth are spherical or ovoid in shape. The cytoplasm is faintly stained by Heidenhain iron haematoxylin. The nucleus is small and spherical. The nucleoli are spherical, small, lying mostly close to the nuclear wall although few ones lie near to or in the central region of the nucleus. The nuclear wall is thickened in some parts due to close adherence of chromatin material. Follicular epithelial cells are isolated, small and compressed and their nuclei occupy the greatest part of the cell.

With the enlargement in size of the oocyte, there is change in its affinity towards dyes. The most characteristic fature of this stage is the formation of the "yolk nucleus". This structure can be distinguished in the materials fixed in Bouin and stained in Heidenhein iron haematoxylin and it is possible to follow the different stages showing the fate of the yolk nucleus. It first appears as a small darky staining spherical structure in close adherence to one side of the nucleus (Fig. 6). The cytoplasm is darkly stained. The nucleus is spherical. The nucleoli are small ; spherical and lie mostly near the nucleus wall or close to it. The nuclear wall is thickened in some parts due the intimate adherence of some chromatin granules. The oocyte wall is still indifferentiated. The follicular cells are compressed, elongated and form a layer around the oocyte. The nuclei of these cells are elongated.

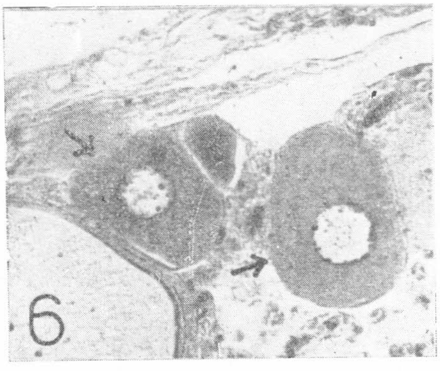

Fig. 6.—Early stage of yolk nucleus formation (450 X).

Yolk nucleus forms a darkly staining cap on one side of the nucleus in the oocytes which is about 60 u. $\times$ 40 u. along its long and short axes respectively (Fig. 7). This organelle encloses the nucleus in the larger oocytes which are about 125 $\times$ 80 u.

On further growth the egg, the yolk nucleus separates from the nucleus and forms a darkly staining network (Fig. 7).

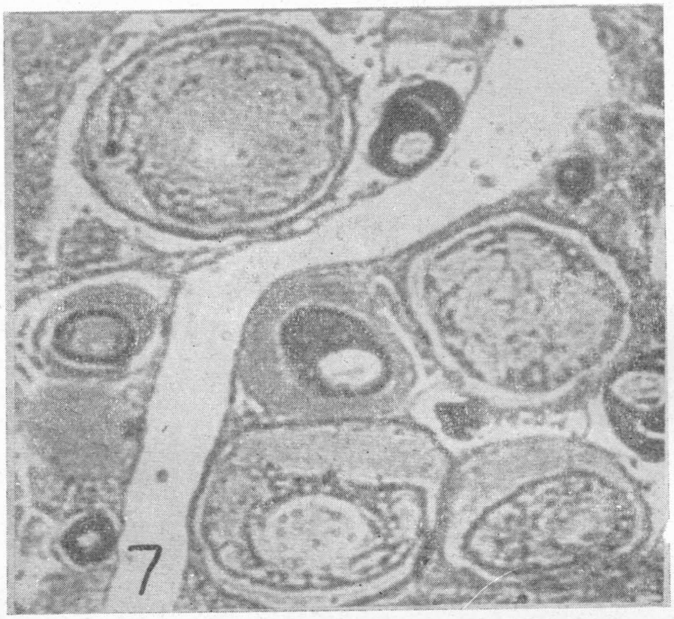

FIG. 7.—Oocytes with different stages of yolk nucleus (270 X).

In the final stage, the yolk nucleus which now becomes faintly stained (Fig. 7), lies in the peripheral region of the cytoplasm of the oocyte which is about 200 × 130 u. Nucleus is 65 ×45 u. The nucleoli are small sperical and lie mostly near or close to the nuclear wall.

In the peripheral zone of the cytoplasm of the oocyte, the yolk nucleus disintegrates and finally disappears and becomes dispersed in the peripheral region of the oocyte cytoplasm. This region thus takes slightly more stain than the remaining part of the cytoplasm which is in this stage very faintly basophilic (Fig. 8). The oocyte reaches a size of 250 × 150 u. along its long and short axes respectively.

The wall of the oocyte is in this stage completely formed and built up of a hardly detectable zona radiata, external to which lies follicular epithelial layer. This is in turn coated by a connective tissue layer.

In the final stages of the protoplasmic growth, the oocyte becomes about 300 × 200 u. and is faintly basophilic. The nucleus is oval or tends to be irregular and has diameters about 90 and 60 u. along its long and short axes respectively. The chromosomes lie in the central region of the nucleus (Fig. 8).

FIG. 8.—Last stage of protoplasmic growth (90 X)

4. *Period of trophoplasmic growth* :

The beginning of this period is distinguished by the formation of marginal vacuoles. The vacuoles first appear in oocytes which are about 310 and 280 u. along the long and short axes respectively. These vacuoles are at first few but as growth of the egg proceeds they extend to cover the whole cortical region of the oocyte cytoplasm. At first the vacuols form a single row which forms a ring in the peripheral region of the cytoplasm but later on the vacuoles increase in number so that more than one row can be distinguished. In fixed materials of *T. nilotica,* the vacoules were found empty and in no case were we able to reveal any content whether Heidenhain iron haematoxylin or Heidenhain Azan techniques was adopted. By the end of the vacuolization stage, the oocytes are about 420 × 280 u. The nucleus is central, oval, faintly irregular in outline and 120 × 100 u. The nucleoli are mostly spherical. Some nucleoli lie close to or near the nuclear wall while others lie in or near the central region of the nucleus. The cytoplasm is faintly basophilic. The wall of the oocyte is formed of zona radiata, 1 — 2 u. thick. This layer is coated externally by a follicular epithelial layer, about 7 u. thick and this is again encircled by a thicker layer of connective tissue which is clearly distinguished by Azan Heidenhain stain.

Yolk deposition first appeared in the oocytes about 435 × 310 in dimensions and in the peripheral region of the cytoplasm. In the early stages, yolk appears as fine granules detectable only by the high power.

By the appearance of yolk, the outer region of the cytoplasm becomes more darkly stained than that lying inwards or around the nucleus. On the whole, the external marginal region of the cytoplasm is free from yolk deposition and in this region lie the marginal vacuoles.

Further growth of the egg is accompanied by centripetal deposition of yolk (Figs. 9 & 10) until the whole cytoplasm becomes filled with yolk. The yolk granules are by this stage larger than those lying inwards of near the nucleus. Oocytes are by this stage 490 × 380 u. The nucleus is faintly basophilic 145 × 100 u. and the nucleoli are larger than in the preceding stage. In some oocytes, the nucleoli are partly close to the nuclear membrane and partly in the central region of the nucleus. In still other oocytes the nucleoli lie in the central region of the nucleus, whereby, the nuclear wall becomes free from nucleoli. Zona radiata is 4 u. thick and has undetectable radial configuration. Follicular epithelium is 9 u. thick. The side walls of these follicular cells are undetectable and their nuclei are spherical and stain darkly.

By the increase in size of the oocyte (Figs. 11 & 12) the outer layer of cytoplasm becomes thin, closely lying inner to the zona radiata and more yolk is deposited. Besides, the size of the yolk globules increases and among the outer yolk globules lie comparatively large empty vacuoles. The increase in site of the egg is accompanied by an increase in the irregularity in the shape of the nucleus. There is a tendency for the migration of the nucleoli to the central region of the nucleus, whereby the nuclear wall becomes free from nucleoli.

Besides, by Sudan III technique, it was revealed that fat is formed in the outer region of the egg and proceeds until it covers the different regions of the cytoplasm and on the whole fat deposited is much lower in amount than the yolk. In the large eggs, yolk globules give a positive Sudan III reaction and this indicates that yolk deposited in the egg is a lipoprotein.

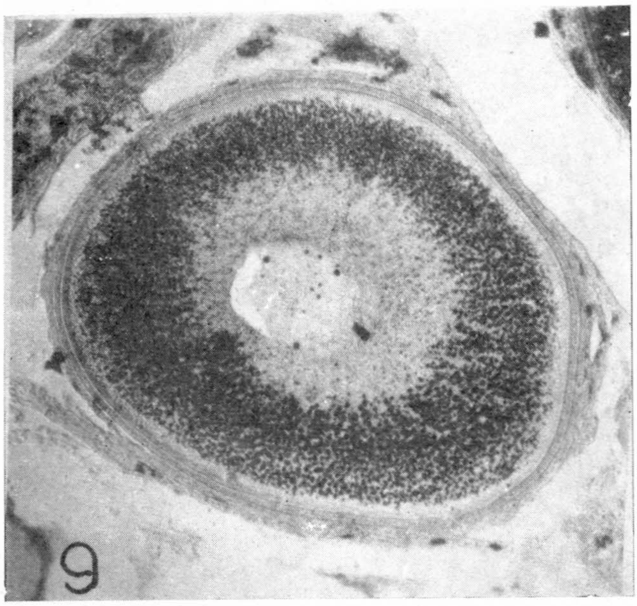

FIG. 9.—Appearance of yolk in outer region of oocyte (90 X).

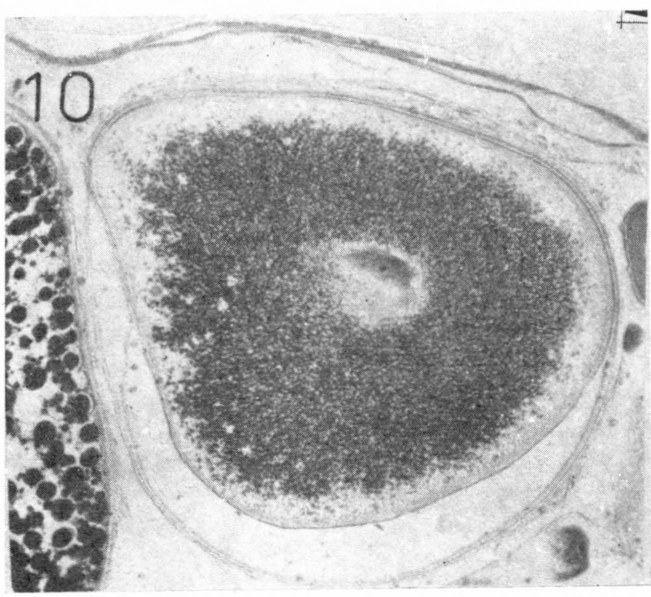

FIG. 10.—Progress of yolk deposition towards centre of oocyte (90 X).

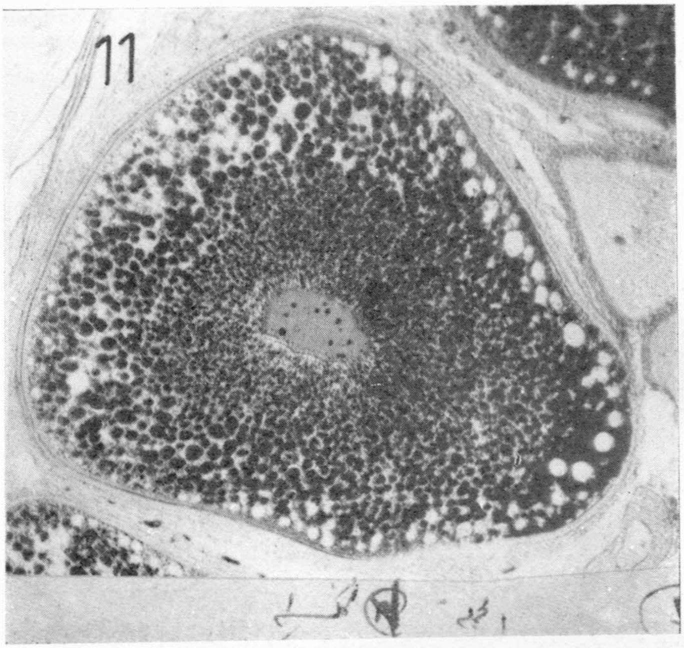

FIG. 11.—Advanced stage of yolk deposition, yolk globular in outer region and granular in circum-nuclear region (90 X).

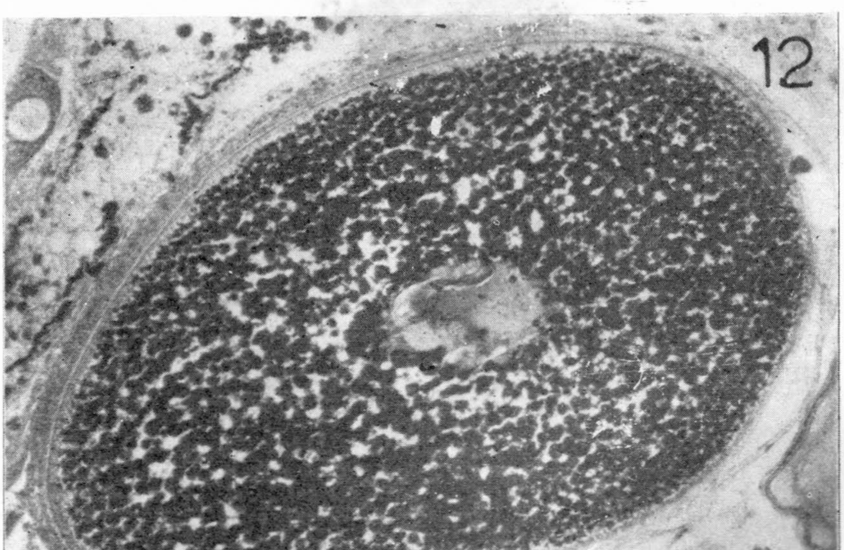

FIG. 12.—Oocyte, more advanced than before, highly distended with yolk globules (90X).

5. *Period of ripening :*

Under suitable conditions, the oocytes which have finished the period of trophoplasmic growth pass into the period of ripening. The first sign of entry of oocytes into ripening is the migration of the nucleus from the centre of the egg towards the periphery. The oocyte by this stage is distinctly pear-shaped, 1200 × 1000 u. along its long and short axes repectively (Fig. 13). The nucleus is irregular in outline. The nucleoli lie in the central region of the nucleus. The migration of the nucleus takes place towards the base of the pear and this shows that this base of the pear forms the animal pole, while the tip of the pear is the vegetative pole. The yolk completely fills the oocyte with a very thin peripheral layer of cytoplasm. The yolk globules are comparatively large, the largest of which are about 30 u. in diameter. The zona radiata is 4 u. thick and the follicular epithelium is about 10 u. thick and surrounded by a connective tissue coat.

FIG. 13.—Oocyte in period of ripening, nucleus eccentric (36 X).

On further progress of the oocyte towards ripening the egg increases in size, and the nucleus comes nearer to the animal pole. The cytoplasm collects to form a coat round the yolk (Fig. 14). In the egg whose dimensions are about 1750 and 1300 u. along the long and short axes respectively, the peripheral layer of cytoplasm is 24 u. The nucleus is amoeboid in shape, 160 and 120 u. along the long and short axes respectively. The nuclear wall is free from nucleoli and the chromosomes become considerably nearer together so that they form a distinct structure which lies in the centre of the nucleus and which is more deeply stained than the holding nuclear material. The yolk globules become larger and more voluminous than in the preceding stage. The largest yolk globule is 50 u. thick. These globules are intermingled with empty vacuoles of varying shape and on the whole they are larger than those of the same principle mentioned above. Besides ,the micropyle is a well differentiated structure in the middle region of the broad base of the egg.

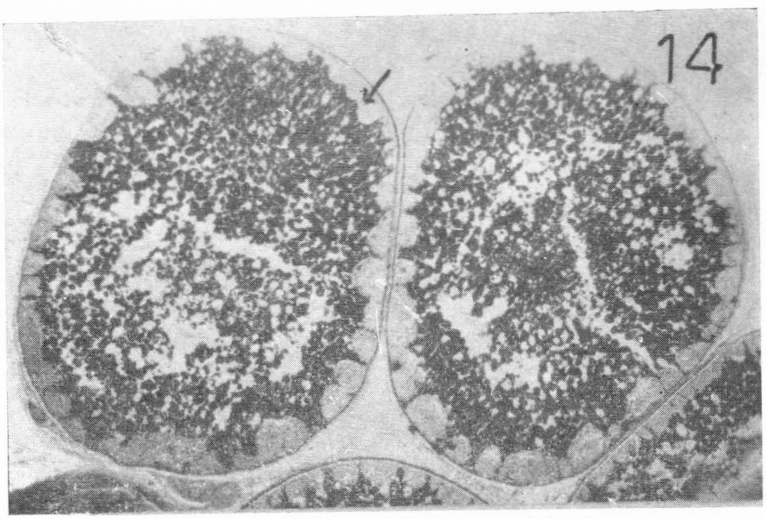

FIG. 14.—Ripening oocyte, cytoplasm forms ring in peripheral region (27 X).

IV.—DISCUSSION

In the ovaries of bony fishes, there is always a reserve of young eggs from which the mature eggs for future generations or spawns are derived. Concerning the origin of these young eggs, mitotic division of the primary germ cells, oogonia and spermatogonia was described in the immature individuals (Bohi, 1904, Bochman, 1914, Hann, 1927, etc..). On the other hand, in the sexually mature females, while some authors could not reveal the mitotic division of oogonia, others observed dividing oogonia (Eggert, 1931; Kazansky, 1949; etc.). According to Eggert (1931), the mitotic division of oogonia proceeds in the course of a very short period following the spawning season. However, in the present species, dividing oogonia were only revealed during September, a fact which may show that the division of oogonia may have a seasonal character. The possibility of division can also be deduced from the fact that during this month oocytes, with 2 nuclei, can be detected as well. However, we cannot conclude whether this character is due to amitotic or mitotic division.

The course of development of the egg is divided into stages, phases or periods in order to differentiate the gradual changes in its conditions or peculiarities. Thus, Bullough (1939) distinguished two phases, a primary and a secondary growth phase, yolk deposition taking place in the latter phase. Futher stages were distinguished by Yamamato (1956 a) who described eleven stages in the course of egg development, yolk deposition covered four of them, viz., yolk vesicle stage, primary yolk stage, secondary yolk stage and tertiary yolk stage. Besides, according to Kazansky (1949), Lapitsky (1949) and others, the process of oogenesis in fishes is distinguished into four periods, viz. 1) period of synapsis, 2) period of slow growth or protoplasmic growth 3) period of intensive growth or trophoplasmic growth and 4) period of ripening.

During the period of protoplasmic growth an important structure is formed to which the names archoplasm, centrosphere, corps vitelline and yolk nucleus (Y. N.) were given.

In *T. nilotica*, this structure first appears as a small organelle closely contiguous to the nucleus. It migrates to the peripheral region of the occyte where it disintegrates and in occytes measuring aout 250 × 150 u. only traces of it are seen.

According to Narain (1937), Chaudhry (1952), and others the Y. N. is made up of the Golgi bodies and nitochondria. Furthermore, Narain (1937) dealing with ***Ophiocephalus, Saccobranchus, Clarias*** and ***Anabas*** believed that the fomation of fatty yolk is related to the Golgi apparatus. Wheeler (1924) mensioned (p. 657) that "Yolk formation is intimately connected with Golgi bodies. This structure plays a leading part in the chemical changes resulting in yolk formation, if indeed it is not itself converted into yolk". In the peripheral region of the egg of *Tilapia nilotica* and where distintegration of yolk nucleus occured, yolk deposition took place. Besides, by using Sudan III, yolk showed a positive reaction revealing the presence of fatty yolk in the egg of the species under examination. It is therefore possible to assume that as was formerly maintained by Narain and others, the yolk nucleus may be related to the formation of fatty yolk. However, presence of the albuminous yolk, of Hibbard and Parat (1927, 1928), Narain (1930, 1937) and others, needs further examination in the eggs of ***Tilapia nilotica.***

The period of trophoplasmic growth begins with vacuolization followed by deposition of yolk and possibly also fat. The content of vacuoles lying in the cortical region of the egg is carbohydrate in the nature (Kusa, 1953 — 1954; K. Yamaoto, 1955 b.; etc.).

In *Tilapia nilotica,* the vacuoles are formed in a manner comparable to that formerly described by many authors and we are unable to detect any content in the cortical vacuoles. However, Bouin was used as fixative by many workers as is also case in the present work. By using Sudan III technique fine "fat" granules were detected in the early stages of vitellogenesis of the egg of *T. nilotica* and it is possible that the fat content was dissolved on staining leaving empty vacuoles.

In *T. nilotica,* yolk was deposited first as yolk granules in the peripheral region of the cytoplasm and then extended centripetally throughout the whole cytoplasm. Such a process of yolk deposition was observed in many fishes e. g. ***Pleuronectes limanda*** (Wheeler, 1924), ***Gasterosteus aculeatus*** (Singh and Boyle, 1940) *Lucioperca lucioperca* (Trusov 1947), etc.

On further growth of the oocytes more yolk is added and this is accompanied by the appearance of yolk globules as well as an increase in the size of the egg. On ripening of the egg, the yolk globules get larger so that in an egg measuring 1750 u. and 1300 u. along the long and short axes respectively, globules up to 50 u. in diameter were recorded.

With the increase in size of the egg, the cytoplasm increases in size to a greater extent than the nucleus. Thus, while in the early stages of growth the nucleus forms a considerable part of the size of the oocyte, in the ripening stage the nucleus occupies a considerably small part of the egg. This is clear from the following table in representative eggs from some growth stages of oocyte.

With the growth of the oocyte, its wall is completely formed. Thus, while in the early phases of protoplasmic growth period, only follicular epithelial cells are distinguished, by the end of this period, the wall of the egg is made up of hardly detectable zona radiata, external to which lie follicular epithelial and connective tissue layers. On further growth of the oocyte, the thickness of the zona radiata increases from 1 — 2 u. in early trophoplasmic growth stage to about 4 u. by the end of this period or in the ripening period. On this account, the zona radiata of the eggs of *T. nilotica* is much thinner than in other species such as Volga sturgeon, *Osmerus eperlanus eperlanus, Esox lucius* (Sadov, 1963) and *Acerina cernua* (Latif, 1966) where the zona radiata is 25.31 u., 15 u. 9.5 u., 13 u. thick respectively. It is possible to assume that the thin zona radiata of the eggs of *T. nilotica* is related to the mouth-breeding habit where the egg is protected. Besides, due to the thickness of the zona radiata the exchange of gases during embryogenesis can proceed well even in spite of the close adherence of the eggs in the buccal and pharyngeal cavities of the female during incubation.

Percentage of diameter of nulens to that of oocytes of different sizes

Period or stage	Diameter (u)		Average of long & short axes (u)		%diametre of nucleus to oocyte
	OOCYTE	NUCLEUS	OOCYTE	NUCLEUS	
Presynaptic stage	12	10	—	—	83.3
End of protoplasmic growth . .	300 & 200	90 & 60	250.0	75.0	30.0
Trophoplasmic growth period					
Vaculolization .	420 & 280	120 & 100	350.0	110.0	31.4
Yolk deposition.	490 & 380	145 & 100	430.0	122.5	28.4
Ripening period .	1730 & 1300	160 & 120	1515.0	140.0	9.2

V.—SUMMARY

1. In sexually mature females, oogonia multiply by mitotic division during September.

2. Stages of growth of egg include periods of synapsis, protoplasmic growth, trophoplasmic growth and ripening.

3. Pre-synaptic oocytes are small, about 12 u. in diameter. Nucleus is about 10 u. Post-synaptic oocytes are 30 u. in diameter, chromosomes are in form of fine coarse threads scattered within nucleoplasm.

4. In protoplasmic growth period, increase in size is due to increase in size of protoplasm. On growth of oocytes, basophility decreases and in final stages, occytes are about 300 u. by 200 u. and faintly basophilic.

Yolk nucleus is formed in this period, first appears as a cap close to nucleus and later forms a ring round it. Y. N., forming a network, migrates to peripheral region of oocyte and finally disintegrates. These stages are accompanied by increase in size of oocyte.

5. In trophoplasmic growth period, peripheral region of cytoplasm first becomes vacolated. Oocytes are about 420 u. by 280 u. in diameter.

Yolk granules first appear in peripheral region of oocytes about 435 by 310 u., extend towards central region thus covering whole ctyoplasm in oocytes about 490 u. by 380 u. Yolk deposition proceeds causing enlargement of egg with formation of yolk globules. Yolk desposited is a lipoprotein and shows positive reaction to Sudan III.

6. In ripening period, nucleus migrates to animal pole or base of pear where micropyle lies. Fixed and stained egg is about 1730 u. by 1300 u.

On further migration, egg increases in size, cytoplasm collects in peripheral region and yolk globules coalesce becoming larger.

7. Wall of egg is formed of thin zona radiata, 4 u. thick, which within ovary is coated by follicular and connective tissue layers. Thin zona radiata was considered as an adaptation to mouth-breeding habit.

8. Early during trophoplasmic growth, nucleoli begin migrating towards central region of nucleus.

VI.—ACKNOWLEDGMENT

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