THE DEVELOPMENT OF THE OOCYTES AND SEASONAL CHANGES IN THE OVARY OF THE IDE IDUS IDUS (L.) IN THE RIVER KÄVLINGEAN, SOUTH SWEDEN [1].

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INTRODUCTION

The structure of the ovary of several teleosts has been carefully described, but no descriptions of the structure of the ide ovary have come to my attention.

The main porpose of the present investigation were to study the development of the oocytes and seasonal changes in the ovary of the ide, in the river Kävlingean in Southwestern Sweden with regard to age and length.

Material and Methods

The material was collected in the lower part of the river Kävlingean from April 1966 to April 1967. The age of the ide was determined by examination of the scales. The total length of the fish (tip of the nose to tip of the tail, lobes compressed) was determined to the nearest millimeter.

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The fish were either held alive (< 36 hours) until used or dissected immediately. The gonads from the immature ide were removed and fixed *in toto* in Bouin's fixative (25 parts sat. aqueous picric acid, 5 parts 40% formalin, 1 part glacial acetic acid). Of the ovary from mature fish or fish going to spawn in the next season only part was fixed. Young-of-the-year ide were fixed *in toto* in Bouin's fixative, but the largest specimens were opened ventrally.

The material was washed several times in 70% ethanol and stored in 70% ethanol. Sections of about 5 mm in length for the production of 10 slides, approximately 15 sections per slide, were taken from one of the anterior, central and posterior portions of the ovary of each fish, except fry shorter than 44 mm from which sections were taken through the whole body. The sections were dehydrated in tetrahydrofuran (C_4H_8O) and embedded in paraffin. Sections were cut serially at 8 μ m and stained with hematoxylin and eosin, and with azan.

Nuclei, the diameter of oocytes and oogonia, were measured using a micrometer eyepiece. Sample size was 20 in all cases. Measurements of oocytes and nuclei were taken from the largest section of the structure in the series of sections. Where the oocytes or nucleus were oval in outline, the parameter recorded was the square root of the product of the greatest diameter multiplied by the least diameter (Lewis and McMillan 1965).

In this account the development of the oocytes of the ide has been arbitrarily divided into five stages in the immature fish and three in the mature specimens.

The general structure of the ovary

The ovaries are paired, extending back from a blunt anterior tip to join at a point just prior to oviduct. The ovarian cavity is composed of transverse lamellae. The entire body cavity of the spawning, phase ide, except for the space occupied by the gut, kidneys, liver, and swim bladder, if filled by the ovaries which may weigh from 87.5 g for a female spawning for the first time, with a body weight of 426 g and being 32 cm total length, to 713 g for an older female having a total body weight of 2,325 g and being 53 cm long, and decrease to below 50 g after the eggs were shed. These data were obtained from about 40 females prior and after the spawning in 1967.

Immature ide

STAGE 1. No appearance of gonad in fry up to about 50 mm in total length or after the first summer of its life (Fig. 1).

STAGE 2. *Undifferentiated gonad.* The sexually undifferentiated gonad is a pair of strings located in the body cavity ventral to the kidneys, lateral to the swim bladder, and are firmly attached to the body wall by means of the mesovarium. The gonad consists of different types of cells (Fig. 2).

STAGE 3. *Oogonia and early oocytes.* The oogonia are medium-sized mean 11.8 μm, spherical cells with large round nuclei, mean 8.6 μm and without visible nucleolus. There is a fairly basophilic cytoplasm and the cell membrane is indistinct (Fig. 3). Oocytes were on average 32.4 μm in diameter and the nucleus had a mean size of 21.9 μm. The nucleolus is difficult to observe. The oocytes are enlarging and beginning to acquire finer basophilic granules in their cytoplasm (Fig. 3). A dense body appear in the cytoplasm which later disappears with the growth of the oocyte (Fig. 4).

STAGE 4. *Basophilic oocytes with large nucleoli occur.* In the beginning the oocytes are on average 90 μm in diameter with a nucleus on average 47.3 μm. One or several nucleoli are found at the periphery of the nuclear membrane (Fig. 5). At the end of the stage when the oocytes have reached a diameter of on average 207.4 μm and the nuclei 107.6 μm, the nucleoli have increased in number and diminished in size (Fig. 6). The oocytes are considerably larger than the oogonia. As the oocytes grow they separate and start to form the lamellae (Fig. 7).

STAGE 5. *The beginning of vitellogenesis.* Yolk first appears in oocytes of on average 250.2 μm in diameter, whose nuclei measure on average 102.8 μm. The primary yolk globules appear in a ring within the cytoplasm rather near to the cell membrane (Fig. 8). The globules are at first clear and colorless, but they begin to turn acidophilic by the time a second layer of yolk globules appears. In these oocytes a thin vitelline membrane occurs around the ooplasm (Fig. 9). At the beginning of vitellogenesis, fine granules begin to accumulate immediately beneath the membrane, forming a very dense granular cortical layer (Spek 1933); beneath this layer there is a zone containing numerous vacuoles. The cortical layer gradually becomes thicker, perhaps at expense of the outher layers of vacuoles (Arndt 1956), and a radial striation becomes visible in it. The number of vacuoles decreases towards the end of oogenesis, so that finally only one layer of cortical alveoli remain just beneath the zona radiata. Successive layers of yolk globules form centrally from the primary layer.

Mature fish

STAGE 6. *Maturing oocyte.* The nuclear membrane desintegrates and the nucleoli disappear. The cytoplasm is more or less filled with yolk

globules (Fig. 10). Early in this stage (in July) the oocytes have a mean diameter of 0.85 mm in fresh conditions, then oocytes mature very rapidly. This maturation is characterized by extensive yolk deposition accompanied by considerable increase in size. A rapid thickening of the zona radiata occurs; measuring 6 μ m in July. Thus, fish with oocytes in stage 6 are mature, i.e. they will oviposit in the next spawning season.

STAGE 7. *Mature oocytes present.* The oocytes are almost as large as ripe eggs, i.e. 1.48 mm in diameter, but whereas the yolk is eosinophilic numerous vacuoles appear in the yolk-filled cytoplasm just beneath the zona radiata (Fig. 11). The nucleus starts to become eccentric and smaller in relation to the size of the oocyte. The zona radiata measures 18 μ m in thickness with typical radial strations. Outside the zona radiata there appears a special "apparatus for fastening the eggs" (Raven 1961), consisting of long threads (Fig. 12). This layer grows around the maturing oocyte and remains to the end of the oogonesis. A single layer of follicular cells surrounds the oocyte. During this stage the yolk platelets become more and more densely packed and enlarged (Fig. 13).

STAGE 8. *The ripe egg.* Living ripe eggs are relatively strongly adhesive. They are spherical, about 1.7 mm in diameter, transparent, and are covered with a thick egg membrane. After ovulation the follicular layer and the surrounding stromal connective tissue collapse to form the lumen which contains cellular debris (Figs. 14 and 15).

The annual cycle of oocytes

Mature fish. Oocytes of stages 3 to 5 can be found in the ovaries of mature fishes throughout the year. The oocytes of advanced stages, however, are observable in ovaries for different lengths of time in various seasons, some stages being found for a few months and others for a few days only. Oocytes corresponding to stage 6 were observed in ovaries during a period from July to late autumn. In November when the oocytes have attained the size of those prior to the spawning, stage 7 appears. A few days prior to the spawning in river Kävlingean (Cala 1970), the ripe eggs appear. Thus, the ovaries of ide close to the spawning season include oocytes in various stages of development (stage 3 to 5 and ripe eggs).

Immature fish. The oocytes in stage 3 to 4 were found in the ovaries of the immature ide all the year round. The oocytes of stage 5 in the immature fish appear one year before the fish becomes mature and thus one year before the appearance of stage 6. Based on these findings it is reasonable that the adolescent phase reflects the spawning rhythm in mature fish.

Atresia

Not all the ripe eggs are shed during the spawning (Fig. 16). Those that remain in the ovary after spawning undergo atresia. They may have been trapped in the folds of the ovary, may not have been completely discharged from the follicle, or may not have been sufficiently developed for ovulation (Lewis and McMillan 1965). Phagocytes derived from follicular cells (Beach 1959) congregate at the periphery of the oocyte and begin to ingest the yolk (Fig. 17 and 18). This process progresses towards the centre of the oocyte until the yolk is an amorphous mass. As the yolk is removed the vitelline membrane and follicular layer colapse. Finally, the vitelline membrane disappears.

Discussion

The first sign of an undifferentiated gonad appeared, in ide from river Kävlingean, after the first growth season or in fish about six months old. The first oocytes appear during the second summer. Oocytes in stage 5 appear, as a rule, in ovaries of fish age group IV, but sometimes they occur in age group III. This stage in one year develops into stage 6, when the fish is mature and the oocytes start to mature. Thus, the female ide may become mature at an age of four years, usually however, at five years age. Thus, female ide can spawn for the first time in the 5th year of life, but mostly in the 6th. This agrees with the field observations (Cala 1970).

In ide, a few peripheral nucleoli of varying size are present in the early stage of the oocyte (see Fig. 5). The nucleoli increase in number and decrease in size as the oocyte approaches vitellogenesis (vide Fig 6), suggesting subdivision and fragmentation. According to Raven (1961), nucleoli may give rise to several daughter nucleoli by fragmentation or budding. Oocytes with two nuclei occur from time to time during early stages of development (Fig. 19).

The primary egg membrane —the vitelline membrane— is of variable thickness. When the vitelline membrane is thick it may consist of several layers and often exhibits radial stration (Raven 1961). Such a *zona radiata* is very distinct in ide (vide Fig. 12).

Outside the zona radiata a further layer may be formed. Sometimes it has only temporary existence and disappears before the end of oogenesis, in other instances it remains. In the latter case it may consist of a thick jelly (e.g. in *Perca*). In teleosts, the egg membrane may bear villi (e.g. in cyprinoids) or possess a special apparatus for fastening the eggs, consisting of long threads (Raven 1961). In the ide such a "fastening

apparatus" is easily visible especially at the end of oogenesis or when the zona radiata has reached its maximum thickness. It entails an adhesive property of the ripe egg of the ide. The fastening apparatus has been described in other cyprinids, e.g. *Abramis brama* (L.) and *Rutilus rutilus* (L.) (Arndt 1960).

Yamamoto (1956, 1957 and 1958) studied the formation of fish eggs with special reference to the vitellogenesis and the histochemistry of the yolk granules. He classified the eggs in two groups the one with a "continuous mass of yolk" and the other with a "non-continuous mass of yolk". The former has minute yolk globules as found in *Fundulus* (Marza et al. 1937), while the latter contains large yolk globules as reported by Yamamoto (1958) in *Clupea*. The eggs of ide resemble the second group in having large yolk globules or platelets of different size (Fig. 13).

In the post-ovulatory atresia, the peripheral yolk platelets begin to clump and vacuoles appear. The vitelline membrane often persists until both yolk and cytoplasm have been removed and it is difficult to ascertain whether its removal is accomplished by active phagocytosis or dissolution. Atresia in oocytes at early stages was not observed; but it is difficult to recognize in those stages (Zuckerman 1962). Early stages of atresia in basophilic oocytes are difficult to distinguish from yolk uptake (Lewis and McMillan 1965), but it seems to occur in basophilic ide oocytes.

The ide sexes can be distinguished with certainity by examination of the gonads only during and after the second summer or growth season of its life, although the gonads are already formed during the first summer (vide Fig. 2). The relation between oocytes development and the age groups of the ide may be summarized as follows (Table 1).

YEAR 1. At the end of the first year of the fry life the gonads are composed of isolated groups of oogonia or spermatogonia, i.e. the gonad is undifferentiated.

YEAR 2. The ovary is composed of oogonia, new oocytes, and a few slightly basophilic oocytes (stage 3). This stage begins during the second summer of the fish life.

YEAR 3. In the third year the ovary is composed of oogonia new oocytes, and basophilic oocytes (stages 3 and 4).

YEAR 4. At the end of the fourth year of life the ovary is composed of oocytes in stages 3-4 and 5.

YEAR 5. During the fifth growth season the ovary may be composed of oocytes of stage 3-6, i.e. some female ide may lay eggs at an age of 5 years.

From this study it also emerged that all female ide older than age group VI were mature and some became mature in the fifth year.

TABLE 1. The relations between oocyte development and age groups of the ide from river Kävlingean, 1966 - 1967.

Stage of development of oocyte	Length of ide in cm			Age group
	Nº	Mean	Range	
—	2	44	43– 45	0 (July)
—	3	58	57– 59	0 (September)
1	2	64	62– 66	I
3	1	148	—	II
3 - 4 and 5	15	218	163–319	III
3 - 4 and 5	2	247	239–255	IV
3 - 4 and 5	2	369	367–371	V
3 - 5	1	287	—	VI
3 - 5, 6, 7 and 8	8	434	410–456	VII
3 - 5, 6, 7 and 8	2	457	446–468	VIII
3 - 5, 6 and 8	3	474	470–492	IX
3 - 5 and 8	1	498	—	X
3 - 5	2	460	460–460	XI

RESUMEN

El desarrollo de los oocitos y el ciclo anual en el ovario de *Idus idus* (L.) ha sido estudiado en el Río Kävlingean, sur de Suecia, con respecto a la edad y longitud del pez.

El material fue coleccionado en la parte baja del río desde abril de 1966 hasta abril de 1967. La edad de los peces fue determinada por medio de las escamas. Las gónadas de los peces inmaturos fueron extraídas y fijadas en flúido de Bouin. Sólo se fijó parte del ovario de los peces maduros o peces que desovarían en la próxima temporada. Peces en su primer año de vida fueron fijados *in toto* en flúido de Bouin, pero los especímenes más grandes fueron abiertos ventralmente.

El material fue lavado varias veces en etanol al 70% y almacenado en etanol de la misma concentración. Se practicaron secciones de la parte anterior, central y posterior del ovario de cada pez, exceptuando alevinos menores de 44 mm long. en los cuales se hicieron secciones a través de todo el cuerpo. Las secciones se deshidrataron en *tetrahydrofuran* (C_4H_8O) y se montaron en parafina. Se hicieron secciones seriales de 8 μm y fueron teñidas con hematoxilina y eosina, y azan.

Los ovarios son pares, extendiéndose hacia atrás para unirse en un punto justamente anterior al oviducto.

Para este estudio, el desarrollo de los oocitos ha sido arbitrariamente dividido en cinco estadios en los peces inmaturos y en tres en los especímenes maduros.

ESTADIO 1. Gónadas no aparentes en los alevinos hasta de *ca.* 50 mm de longitud total, o después del primer verano de vida (Fig. 1).

ESTADIO 2. *Gónada indiferenciada.* Consta de diferentes tipos de células (Fig. 2).

ESTADIO 3. *Oogonios y oocitos primitivos.* Los oogonios son de tamaño medio (promedio 11,8 μ m) esféricos con núcleos grandes redondeados (promedio 8,6 μ m) y sin nucléolo visible. La membrana de la célula es indistinta (Fig. 3). El diam. de los oocitos mide en promedio 32,4 μ m y el núcleo tiene un tamaño promedio de 21,9 μ m. Los oocitos están en crecimiento y empiezan a adquirir granos basofílicos más finos en su citoplasma (Fig. 3).

ESTADIO 4. *Oocitos basofílicos con nucléolos grandes.* Inicialmente los oocitos miden en promedio 90 μ m en diam. Uno o varios nucléolos se hallan en la periferia de la membrana nuclear (Fig. 5). Al finalizar el estadio, cuando los oocitos han adquirido un diámetro promedio de 207,4 μ m y los núcleos 107,6 μ m, los nucléolos han aumentado en número y disminuído en tamaño (Fig. 6).

ESTADIO 5. *Comienzo de la vitelogénesis.* El vitelo aparece primero en oocitos de un diámetro promedio de 250,2 μ m, cuyos núcleos miden en promedio 102,8 μ m. Los primeros glóbulos de vitelo se disponen en un anillo dentro del citoplasma relativamente cerca de la membrana celular (Fig. 8). En estos oocitos una membrana vitelina delgada aparece alrededor del ooplasma (Fig. 9). El estrato cortical gradualmente se engruesa, y en él se hacen visibles estriaciones radiales. Sucesivamente se forman estratos de glóbulos de vitelo en dirección centrípeta.

ESTADIO 6. *Oocitos en proceso de maduración.* La membrana nuclear se desintegra y los nucléolos desaparecen. El citoplasma está más o menos lleno de glóbulos de vitelo (Fig. 10). Al comenzar este estadio (julio) los oocitos tienen un diámetro promedio de 0,85 mm en fresco, entonces los oocitos maduran muy rápido. Esta maduración se caracteriza por deposición extensiva de vitelo acompañada por considerable aumento en tamaño. Además ocurre un rápido engrosamiento de la *zona radiata.* Así los peces con oocitos en estadio 6 son sexualmente maduros, i.e. desovarán el siguiente período de postura.

ESTADIO 7. *Oocitos maduros presentes.* Los oocitos son casi tan grandes como los huevos listos para ser expelidos, i.e. 1,48 mm diam. El vitelo es eosinofílico y numerosos vacuolos aparecen en el citoplasma, lleno de vitelo, justamente debajo de la *zona radiata* (Fig. 11). La *zona radiata* mide 18 μ m de espesor con típicas estriaciones radiales. Afuera de la *zona radiata* aparece un aparato especial "aparato de adhesión de los huevos" —*Apparatus for fastening the eggs* (Raven 1961)—, consistente en filamentos largos (Fig. 12).

ESTADIO 8. *Huevos maduros o listos para ser expelidos.* Estos huevos en condiciones frescas son fuertemente adhesivos. Son esféricos, *ca.* 1,7 mm diam., y están cubiertos por una membrana gruesa. Luego de la ovulación, los estratos foliculares y el tejido conectivo estromático circundante entra en colapso para formar el lumen que contiene residuos celulares (Figs. 14 y 15).

Así, el primer signo de una gónada indiferenciada aparece, en *Idus idus* (L.) del río Kävlingean, después del primer período de crecimiento o en peces aproximadamente de seis meses. Los primeros oocitos aparecen durante el segundo verano. Oocitos en el estadio 5 aparecen usualmente en ovarios de peces de edad *(age group)* IV, pero algunas veces se hallan en la edad III. El paso del estadio 5 al estadio 6 requiere un año y entonces el pez se considera maduro y los oocitos inician la maduración. Es decir, la hembra de *I. idus* puede alcanzar madurez sexual a una edad de cuatro años, sin embargo usualmente la adquiere a los cinco años de edad, de manera que las hembras pueden desovar por primera vez en el quinto año de vida, pero la mayoría en el sexto.

No todos los huevos maduros son puestos durante el desove (Fig. 16). Los que aún quedan en el ovario después del desove sufren atresia. Las plaquetas periféricas de vitelo comienzan a aglutinarse apareciendo vacuolos. La membrana vitelina a menudo persiste hasta que tanto el vitelo como el citoplasma han desaparecido, siendo difícil determinar si su desaparición es efectuada por fagocitosis o disolución.

La relación entre el desarrollo de los oocitos y la edad del *I. idus* (ver Tabla 1), puede ser resumida como sigue:

AÑO 1. Al finalizar el primer año de vida del alevino las gónadas están compuestas por grupos aislados de oogonios o espermatogonios, i.e. la gónada es indiferenciada.

AÑO 2. El ovario está compuesto por oogonios, nuevos oocitos, y unos cuantos oocitos levemente basofílicos (estadio 3). Este estadio comienza durante el segundo verano de la vida del pez.

AÑO 3. En el tercer año el ovario está compuesto por oogonios, nuevos oocitos, y oocitos basofílicos (estadios 3 y 4).

AÑO 4. Al terminar el cuarto año de vida el ovario está compuesto por oocitos en los estadios 3-4 y 5.

AÑO 5. Durante la quinta etapa de crecimiento el ovario puede estar compuesto por oocitos en los estadios 3 a 6, i.e. algunas hembras pueden poner huevos a una edad de cinco años.

De este estudio también emerge que toda hembra *I. idus*, en el río Kävlingean, mayor que el grupo de edad *(age group)* VI es sexualmente madura y algunas adquieren madurez en el quinto año.

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EXPLANATION OF FIGURES

All photomicrographs are of transverse sections cut at 8 μm and stained with hematoxylin and eosin unless otherwise indicated.

PLATE I

1. Mid section of 32 mm ide fry showing the site for the future pair of gonads ventral to the kidneys and lateral to the swim bladder.
2. Posterior section of gonad of 61 mm ide showing sexually undifferentiated gonad.
3. Anterior region of ovary of 148 mm immature ide showing oogonia and newly formed oocytes.
4. Anterior region of ovary of 225 mm immature ide showing newly formed oocytes with a dense body (D). Stained with azan.

PLATE II

5. Posterior region of ovary of 300 mm immature ide showing basophillic oocytes with a few large nucleoli and many peripheral nucleoli in later stages.
6. Anterior region of ovary of 460 mm mature ide with oocytes showing large nuclei with many diminished peripheral nucleoli.
7. Mid portion of ovary of 371 mm immature ide showing oocytes in lamellae.
8. Anterior region of ovary of 444 mm mature ide with oocytes showing ring of primary yolk globules in the cytoplasm.

PLATE III

9. Section from ovary shown in 8. Yolk globules at early stage of vitellogenesis. Note thin vitelline membrane.
10. Mid region of ovary of 492 mm mature ide showing mature oocytes with desintegrating nuclear membrane and thicker vitelline membrane.
11. Mid region of ovary of 445 mm mature ide showing eosinophilic mature oocytes with ring of vacuoles just beneath the zona radiata. The nuclei are becoming eccentric.
12. View of one mature oocyte shown in 11 showing large zona radiata (R), fastening apparatus (A), and the single layer of follicular cells (F).

PLATE IV

13. Posterior region of ovary of 470 mm mature ide prior to spawning showing the yolk platelets and vacuoles beneath the zona radiata.
14. Posterior region of spent ovary of 498 mm ide showing different groups of immature oocytes.
15. Section from same region as shown in 14 with collapsed stromal connective tissue and lumen (L.).
16. Anterior region of spent ovary of 498 mm ide showing different groups of oocytes. Note that one mature or ripe oocyte has not been shed (O).

PLATE V

17. Anterior portion of spent ovary of 446 mm ide showing one oocyte in atresia.
18. Section from same region as in 17 of one atretic oocyte showing embedded phagocytes (P).
19. Anterior region of ovary of 180 mm immature ide showing an oocyte in early stage of development with two nuclei.

PLATE I

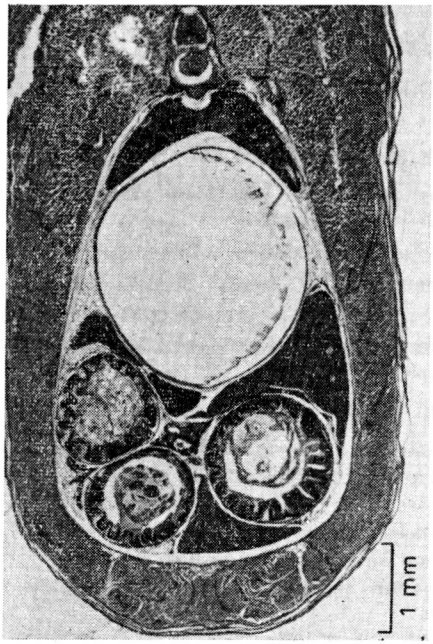


FIGURE 1

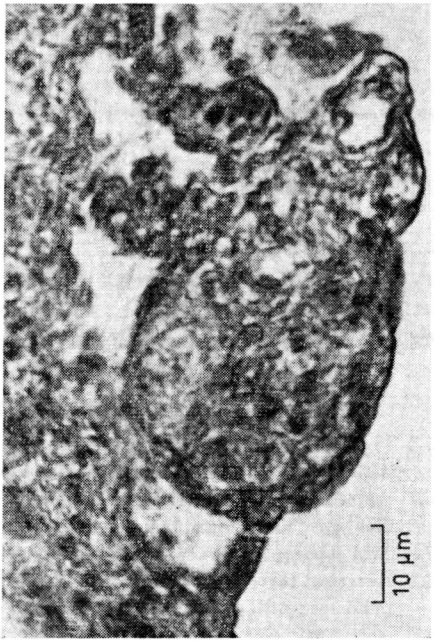


FIGURE 2

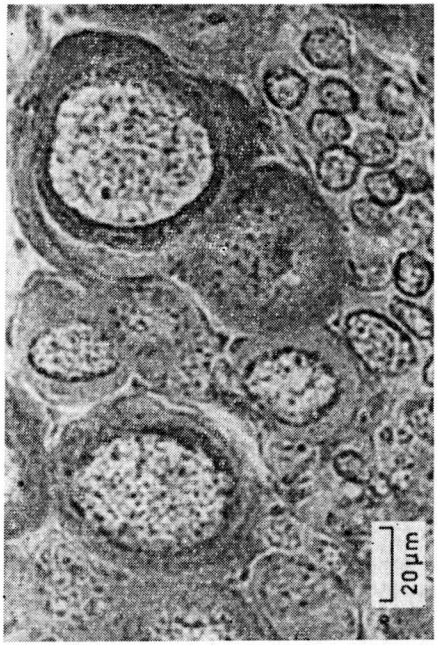


FIGURE 3

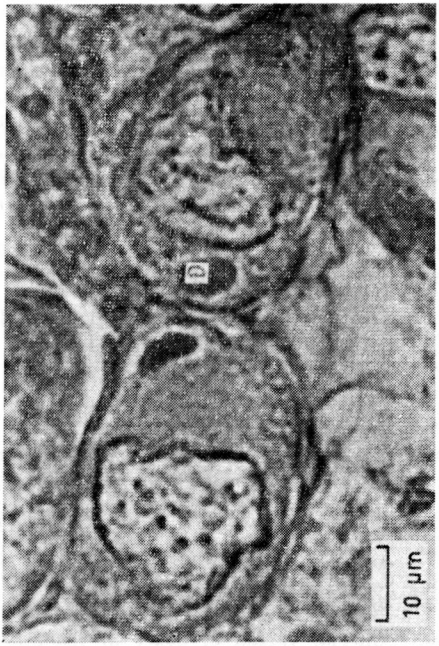


FIGURE 4

PLATE II

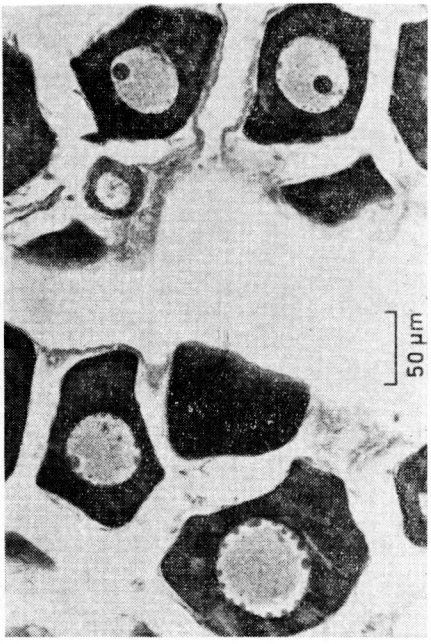


FIGURE 5

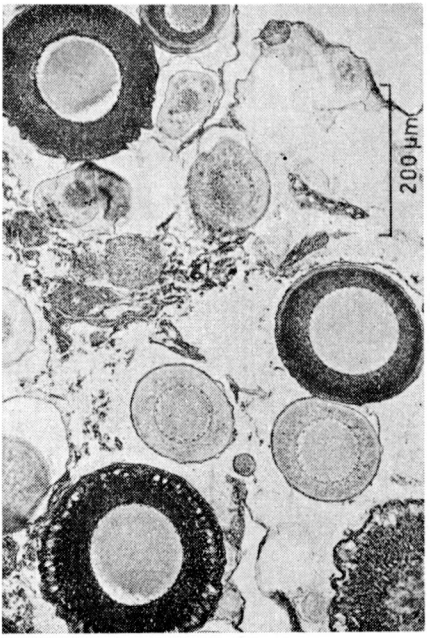
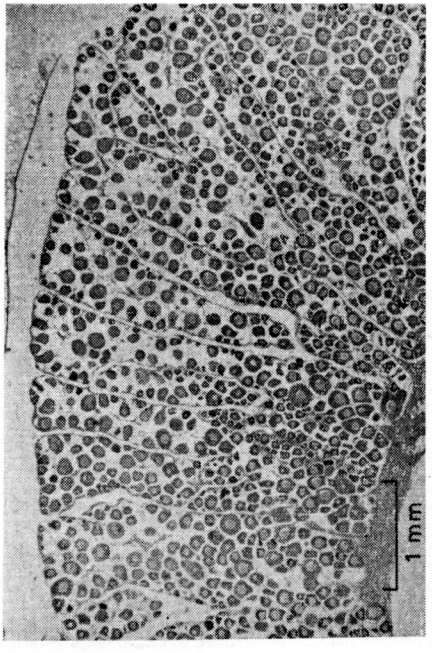


FIGURE 6



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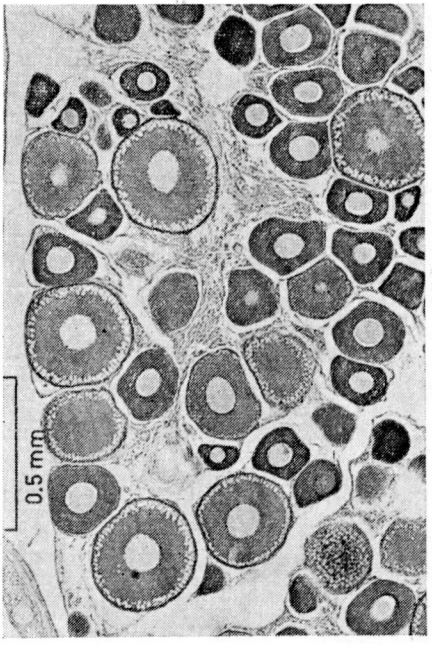


FIGURE 8

PLATE III

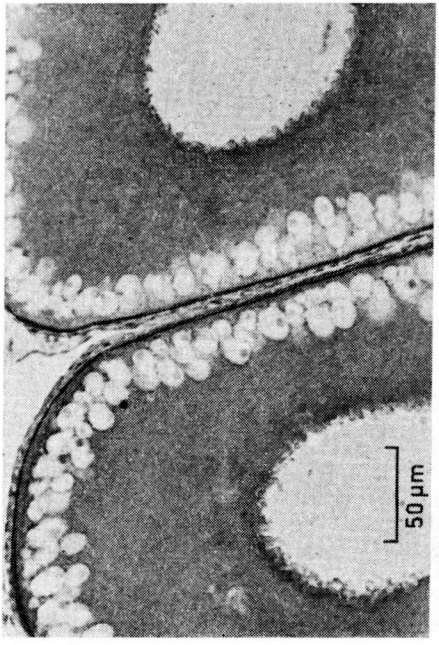


FIGURE 9

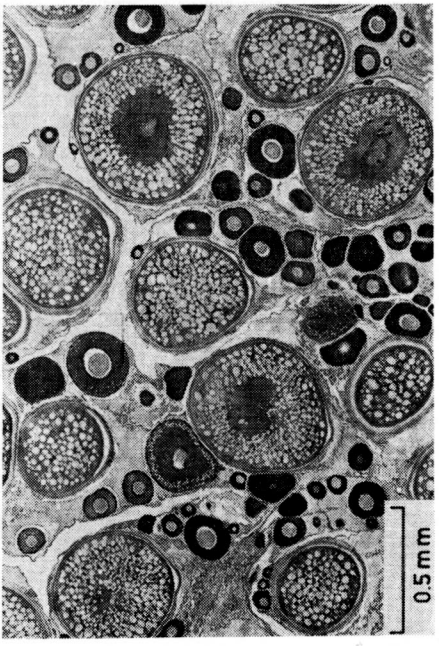


FIGURE 10

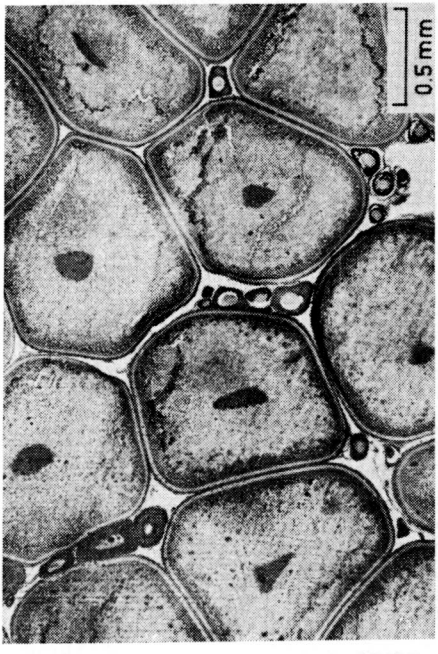


FIGURE 11

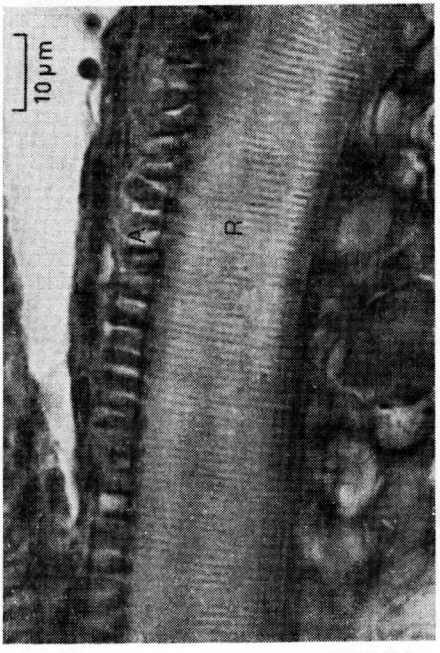


FIGURE 12

PLATE IV

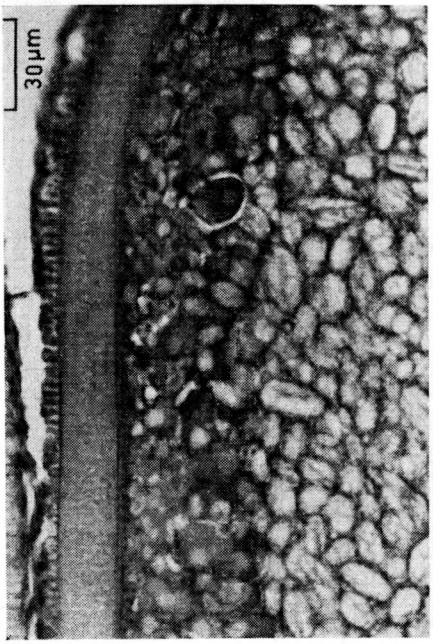


FIGURE 13

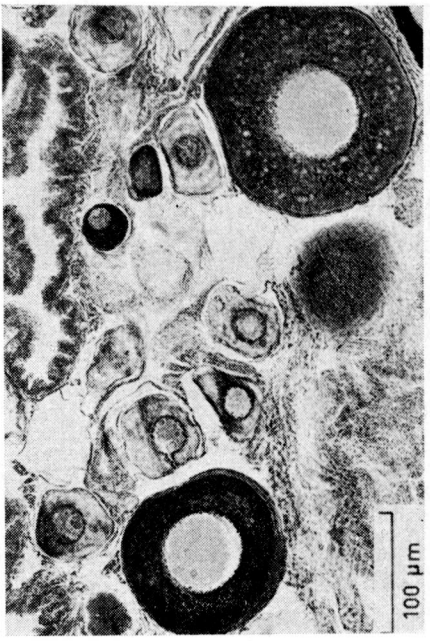


FIGURE 14

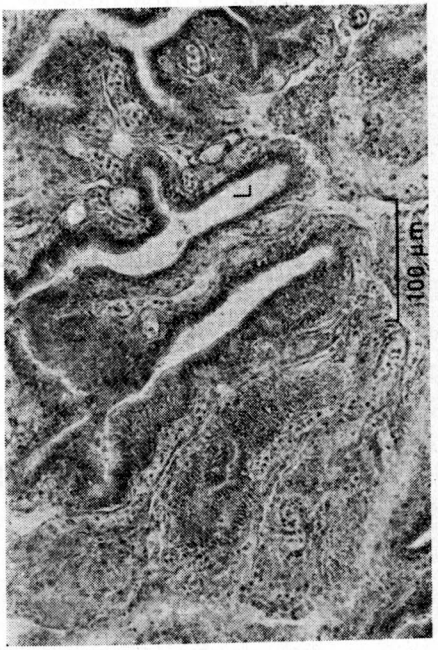


FIGURE 15

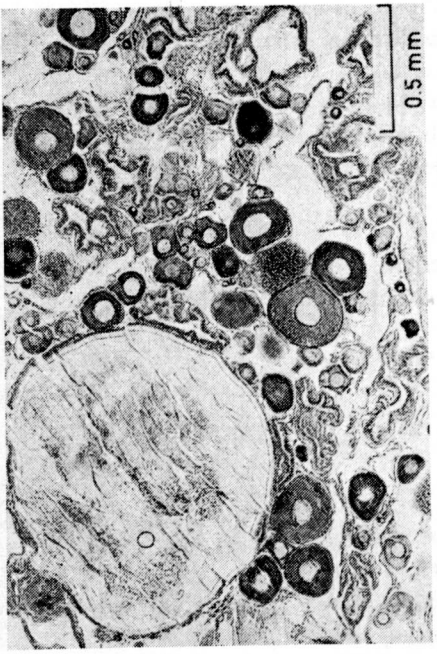


FIGURE 16

PLATE V

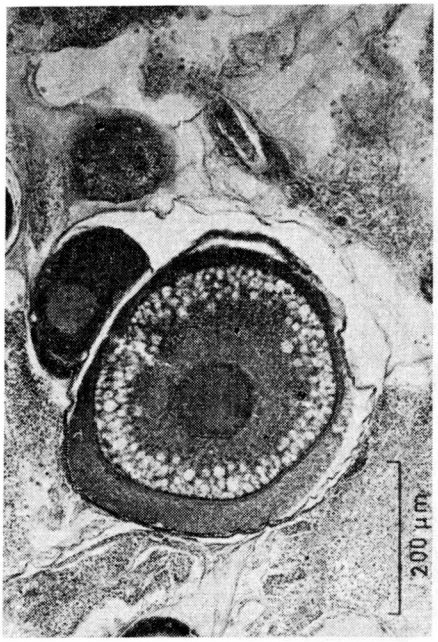
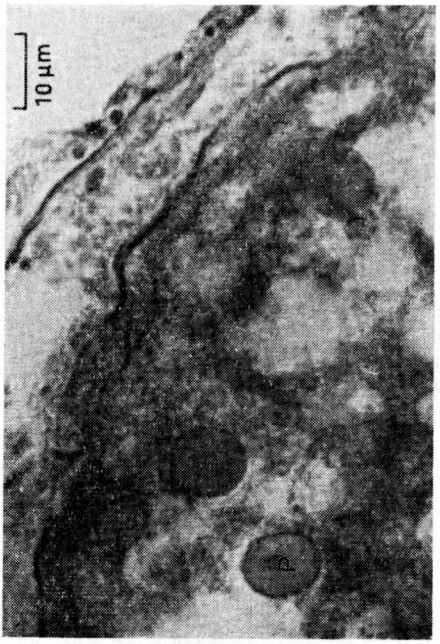


FIGURE 17



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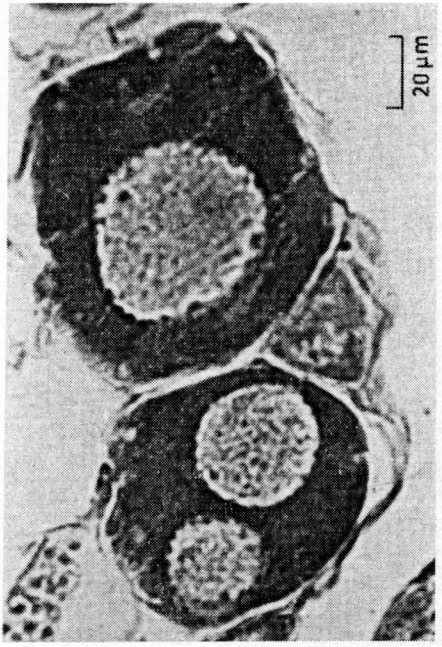

FIGURE 19