CHAPTER III

OVARY

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The Indian wall lizard shows seasonal reproductive cycle. (Mahendra, 1936). The ovaries are situated in the abdominal cavity which during the prebreeding period contain abundent ova with different maturing stages. During breeding period they contain ova which are large in number. Only one ovum at a time is found in the oviducts, of this gecko, which is a pecularity of the geckos (Church, 1962; Jones et al, 1978).

The detailed morphology of H. flviviridis ovary has been studied by Guraya, (1978); Guraya and Varma (1976) (1978); Varma and Gurya, (1975). These morphological studies revealed the ovarian histology, which depicts that the follicular cells wrap the oocyte to form the follicular wall. The outermost layer of the cells form granulosa layer of the oocyte. With the oocyte growth, zona pellucida is formed in between the oocyte membrane and follicular cells. The zona pellucida can be differentiated into the two layers, theca interna and theca externa. The follicular wall exhibits greater development and complexity in saurians. With the initiation of the growth, young oocyte is surrounded by a follicular epithelium which consists of a single layer of flattened follicle cells, having similar morphology. In lizards and snakes they become first unilayered then bilayered and subsequently multilayered, which is also true for H. flaviviridis. In the follicular wall, three types of cells, small, intermediate and large

pyriform can be differentiated, when it is multilayered. In vitellogenic follicles, the pyriform cells become smaller and the follicular epithelium becomes single layered and monomorphic. The atretic follicles in the different phases of degeneration are also found frequently in H.flaviviridis ovary (Gouder and Nadkarni, 1976).

In addition to the above morphological reports, there are few histochemical reports available on H.flaviviridis ovaries. Varma and Guraya (1975); Guraya and Varma (1976), studied the lipids in the maturing oocytes. Saidapur and Nadkarni (1976) studied steroid dehydrogenases in the ovaries. Saxena (1979) studied cortical region of H.flaviviridis oocytes and reported RNA and DNA content of different oocytes and mentioned the PAS reactivity of zona pellucida.

So, from the above mentioned literature, review, it seems that the studies on mucosubstances in H.flaviviridis ovaries has received a poor attention. So the present study is an attempt to study the different mucopolysaccharides in the ovaries and their probable functioning during the maturation of oocytes.

OBSERVATIONS

The females of H.flaviviridis were sacrificed and ovaries were processed as mentioned in the Chapter II.

A) HISTOLOGICAL SEGREGATION:

1) Oocytes: For better analysis of the distribution of mucosubstances in the different oocytes; the oocytes were segregated on the histological basis, mainly the growth and differentiation of follicular wall and its successive degener - ation during vitellogenesis (Guraya, 1978, Tokarz, 1978).

O_1 -stage - The oocytes in germinal bed with thin granulosa layer, unicellular follicular wall and distinct cytoplasm, were segregated under this category (Fig. 1-O_1).

O_2-stage - The oocytes at this stage also showed the same histological characteristics as O_1-stage but the follicular wall was bilamellar, (Fig. 1-O_2).

O_3-stage - 03-stage oocytes had all the features same as in O_2 a nd O_1 stages but the follicular wall became multilayered and thick; but the cells from the follicular wall were monomorphic(Fig.4).

O_4-stage- The histological pattern of these oocytes was same as O_3-oocytes; but they differed in possessing few pyriform cells in the follicular wall (Fig.6).

O_5-stage - The main distinguishing feature of these oocytes was the appearance of maximum number of pyriform cells in the multilayered follicular wall. The granulosa layer appeared some what thick and ooplasm increased in volume(Fig.8).

O_6-stage- The O_6-oocytes showed thick granulosa layer, multicellular follicular wall but the pyriform cells showed degenerating pattern, which was evident by the vacuolar cytoplasm. But the zona pellucida in these oocytes was conspicuous and two layered. The ooplasm showed distinct increase in volume and presence of yolk granules (Fig. 10).

O_7-stage- The histological picture of O_7-stage oocytes was similar to the O_6-stage; but the pyriform cells showed advanced degeneration. The yolky ooplasm showed further increase in volume (Fig. 12).

O_8-stage- These oocytes showed thin layer of granulosa cells completely degenerated follicular wall, well developed zona pellucida and well differentiated cortical region even in eosin-haematoxylene preparation. The ooplasm was full with globular yolk (Fig. 16).

O_9-stage- These oocytes showed all the features as O_8-stage oocytes except that the granulosa layer was more thin and yolky ooplasm was increased tremendously in volume (Fig. 17).

O_6 to O_9 were vitellogenic stages, their vitellogenic nature was confirmed by the simultaneous preparation of Sudan black B by which the yolky ooplasm stained blue black. The O_1 to O_5 stages were previtellogenic stages.

2) Atretic follices: The atretic follicles were also classified in the four stages as described by Saidapur (1978), as per Betz's (1963) classification.

Stage 1 - This stage showed the onset of a disorganization of ooplasm which showed slight vacuolization. The zona pellucida showed partial disintegration (Fig. 18).

In follicles with polymorphic follicular cells, the follicular wall showed increased thickness and vacuolar cells. (Fig. 19,20,21,22).

Stage II- The advanced stage of disorganization is noted at this stage. The zona pellucida showed distinct disorganisation. The follicular cells showed invagination in yolk. The follicle was totally shrunken (Fig. 23-arrow).

Stage III - All the changes observed in stage II became pronounced at this stage. The granulosa cells invaded yolk completely. The folicle was more shrunken, wrinkled and vascular (Fig. 24,25).

Stage IV - The marked degeneration of follicular cells was reported at this stage. The follicle was invaded completely by connective tissue elements. The reduced follicle persisted as a scar of connective tissue (Fig. 26).

B) DISTRIBUTION OF MUCINS:

i) Granulosa layer - The granulose layer was present in all the O_1 to O_9 stages which was some what thick in O_6 and O_7 stages (Fig. 10, 12-G). These granulosa cells in all the stages studied, showed PAS (Periodic Acid Schiff's reaction) reactivity which was sensitive to malt-diastase digestion

and could be blocked by phenyl-hydrazine pretreatment; indicating the presence of neutral mucins and glycogen in them (Fig. 1 to 26G). The outer most layer of follicular wall (F a thin sheet). Table No. 1.

ii) Follicular wall - Follicular wall cells were unreactive to any of the methods employed in O_1 and O_2 stages indicating absence of mucopolysaccharidas in them. In O_3 stage the follicular wall was multilayered but monomorphic (Fig. 2F). Some of the cells from the follicular wall exhibited all the reactions indicating presence of glycogen and neutral muco-substances; while the remaining cells were unreactive to any of the methods used, showing absence of any type of mucopolysaccharides. The distinct reactivity of the some cells to PAS enabled to distinguish them as PAS reactive cells (Fig. 2-arrow); while the remaining cells as PAS unreactive cells (Fig. 2-unstained cells). In O_4 oocytes, the follicular wall cells could be differentiated morpholo-gically into pyriform cells, and intermediate cells, or round cells (Fig. 6-F). The newly appeared pyriform cells showed all the reactions exhibited by neutral mucins and glycogen (Fig. 6-arrow), and the round intermediate cells did not exhibit any of the reactions showing absence of mucopolysaccharides (Fig. 6-Non-stained cells). In O_5 stage oocytes, the follicular wall showed maximum number of pyriform cells and few round intermediate cells (Fig. 8-F).

The pyriform cells showed dense PAS reactivity which was due to the glycogen and neutral mueosubstances (Fig. 9). These pyriform cells showed distinct cytoplasmic projections towards the membrane of the oocyte, these projections also showed presence of neutral mucins and glycogen (Fig. 9-arrow). The follicular wall cells (Pyriform and round intermediate) did not show any reactivity to all the methods employed for the analysis of mucosubstances in O_6 to O_9 stages of oocytes. In the O_6 and O_7 stages they were in degenerating condition (Fig. 10, 12); while in O_8 and O_9 stages they were completely degenerated (Fig. 16, 17), Table No. 1.

Zona Pellucide - The zona pellucida could not be identified in O_1, O_2 and O_3 stages of oocytes. The O_4 stage showed appearance of very thin zona pellucida. At this stage the zona pellucide showed presence of glycogen and neutral mucins (Fig. 6-Z). In O_5 stage also, the zona pellucida was some what thickened, but showed all the reactions exhibited by O_4 stage oocyte (Fig. 8-Z). In O_6 stage the zona pellucida was thick and distinct and could be divided into jnner theca interna and outer these externa. The theca externa showed presence of glycogen and neutral mucosubstances (Fig. 10-Z). In O_7 , O_8 and O_9 stages the zona pellucida persisted; and showed presence of glycogen and neutral mucosubstances, (Fig. 12, 16, 17-Z) Table No. 1.

Peripheral cytoplasm -

The peripheral cytoplasm or ooplasm could not be distinguished in O_1 and O_2 stages. In O_3 stage the peripheral cytoplasm showed initial staining reactions indicating presence of glycogen and neutral mucins, containing vicglycol groups. The moderate alcianophilia at pH 1 and moderate alcianophilia at pH 2.5 indicated the presence of the sulfated and carboxy mucins (Fig. 3-arrow). The sulfomucins were infered from their blue purple staining with AF (Aldehyde Fuschin), AB (Alcian Blue) pH 2.5 sequence, β-metachromasia with azure A at pH 1.5, persistant alcianophilia in the presence of 0.4 M Mg^{++} and loss of alcianophilia in methylated sections which could be restored partially, following saponification. The alcianophilia was partly sensitive to acid hydrolysis, thus confirming the acidic mucins. All these results indicated presence of neutral mucins, glycogen, sulfomucins and acidic mucins in the peripheral region of the cytoplasm of O_3-oocytes. The peripheral region in O_4, O_5 and O_6 oocytes also exhibited all the reactions mentioned for O_3- stage indicating presence of glycogen, neutral mucins, carboxy mucins and sulfomucins. The peripheral ooplasm of O_7, O_8 and O_9 oocytes was unreactive to all the methods employed indicating absence of glycogen, neutral mucins, carboxy mucins and sulfomucins in this region (Fig. 7-arrow), Fig. 10-arrow), Table No. 1.

O_4

Cortical cytoplasm:

The cortical zone of the ooplasm could not be distinguished in O_1 to O_5 stages. In O_6 stage it displayed poor reactivity with PAS and weak alcianophilia at AB pH 1 and AB pH 2.5. In further analysis the presence of poor amount of neutral, carboxy and sulfo mucins were confirmed in this region (Fig. 10-C). All the reactions noted in O_6 stage were found in the O_7 and O_8 stages. The intense reactivity to PAS and an intense alcianophilia at AB pH 1. and AB pH 2.5 showed presence of high amount of the three types of mucopolysaccharides i.e. neutral, carboxy and sulfomucins. In O_8 oocyte the PAS reactivity and alcianophilia at AB pH 1 and AB pH 2.5 showed granular nature (Fig. 16-C G). The PAS reactivity and the site of the granules in cortical region showed their cortical granular nature. These cortical granules contained neutral mucins, carboxy mucins and sulfated mucins. Og-oocytes also showed such type of cortical granules in them (Fig. 17-C G), Table No. 1.

Inner Ooplasm:

The inner ooplasm in O_1 to O_5 stages showed presence of glycogen. O_6 stage did not show any reactivity to the various histochemical methods employed. O_7 , O_8 , and Og-oocytes showed presence of the three types of mucins i.e. neutral, carboxy and sulfomucins. All the above results indicated that the yolky material in ooplasm, contains the small amount of neutral, carboxy and sulfomucins (Fig. 14, 15, 16, 17) Table No. 1.

Atretic follicles:

Granulosa cells - Granulosa cells in stage 1 atretic follicles showed moderate reactivity to PAS which could be blocked by pretreatment of phenyl hydrazine and resistant to maltdiastage digestion, indicating presence of neutral mucins and glycogen. (Fig. 19,20-arrow).

The oocytes with polymorphic follicular wall undergoing degeneration (Stage 1 atretia) showed presence of poor amount of glycogen and neutral mucins.

The granulosa cells of stage II showed poor amount of glycogen and neutral mucins (Table No.2).

The reaction pattern exhibited by stage III atretic follicies was much more similar to that displayed by stage II atretic follicies.

The stage IV atretic follicle showed absence of all the types of mucins in remaining granulosa cells and hence failed to exhibit any of the histochemical techniques employed. (Table No.2).

i) Follicular wall - The follicular wall was only present in stage-I atretia. (Atretic follicles with polymorphic follicular wall). The cells undergoing degeneration showed very poor amount of glycogen and neutral mucins.

Some of the phagocytotic invaded cells in ooplasm showed an intense reactivity to PAS, intense alcianophilla to AB pH 1 and AB pH 2.5 and in further analysis confirmed the presence of neutral, carboxy and sulfomucins in them. (This was noted in stage III Fig.24,25-arrow).

II) Zona pellucida - The disintegrating zona pellucida in stage I showed very poor amount of glycogen and neutral mucins (Fig. 18,22). Same type of results were obtained with atretic follicles with the polymorphic follicular wall. The stages II, III, IV did not show zona pellucida (Fig.23,24, 25,26), Table No.2.

III) Ooplasm - The ooplasm at stage-I atretic follicles showed presence of neutral, carboxy and sulfomucins in them but very poor in amount. (The polymorphic follicular walled atretic follicles showed poor amount of glycogen). The yolky ooplasm could not be identified in stage II and III. It was completely degenerated in the stage IV, (Fig.23, 25, 26) Table No.2.

IV) Connective tissue: This was the specific feature of stage IV. The connective tissue showed moderate amount of neutral, carboxy and sulfomucins in them (Fig.26). Table No.2.

D I S C U S S I O N

The observations revealed the presence of glycogen and neutral mucopolysaccharides in granulosa cells of all the oocyte stages. The follicular wall cells showed appearance of

monomorphic PAS reactive cells at O_3 stage which showed to contain neutral mucins and glycogen; and the other round intermediate cells in the follicular wall did not contain any mucins and remained PAS unreactive. In O_4 oocytes pyriform cells appeared in follicular wall which showed presence of glycogen and neutral mucins exhibiting PAS reactivity. In O_5 stage these cells were maximum and their protoplasmic prolongations towards oocyte membrane were evident. In O_6 to O_9 oocytes the follicular wall cells degenerated progressively and did not contain any type of mucins.

So, the appearance of PAS reactive cells in monomorphic follicular wall; the PAS reactivity of the pyriform cells (which newly appeared in O_4-oocyte), in successive stage of development (O_5-oocyte); their progressive degeneration in the later stages of oocytes (O_6,O_7); and the absence of mucosubstances; in pyriform cells of vitellogenic oocytes (O_6 and O_7) appear to be indications of the transformation of PAS reactive monomorphic cells of follicular wall into PAS reactive pyriform cells in follicular wall, which appeared in the successive stages of development. Such type of transformation of monomorphic follicular wall cells into pyriform cells has been suggested in other lizards (Hubert and Andrivon, 1971; Jones et al. 1975; Tokarz, 1977), with autoradiographic and ultrastructural studies. In present

studies their similar chemical composition (glycogen and neutral mucins) may be indicative of such transformation. The proliferation of the follicular wall cells is under the influence of FSH (Tokarz, 1977). So, the changes occuring in the chemical components of the cells like glycogen and neutral mucins may be indirectly under the influence of FSH as suggested by Tokarz (1977).

The PAS reactivity of zona pellucida was not evident in O_3-oocytes, at O_4 and O_5 stages of development the PAS reactive zona-pellucida was very thin, while in O_6-oocytes the PAS reactive zona pellucida was thick and the PAS unreactive theca interna could be made out. In O_7 to O_9 stages the PAS reactivity of zona pellucida was evident. The number of methods employed for mucopolysaccharide staining indicated the nature of PAS reactive material as glycogen and neutral mucopolysaccharides.

When the appearance of PAS reactive zona pellucida is compared with the histochemical changes taking place in developing follicular wall; it is noted that with the appearance of PAS reactive monomorphic cells in follicular wall in O_3-oocytes, the thin PAS reactive zona pellucida is observed; which showed increase in thickness during O_4 and O_5 stages of oocytes, and became evident at O_6 stage. Simultaneous to this development the PAS reactive material was observed in pyriform cells. In addition the pyriform cells at O_5-stage

showed distinct protoplasmic prolongations towards the membrane of the oocyte. These prolongations were PAS reactive, and the nature of this PAS reactive material is of glycogen and neutral mucin type. So, the concurrent appearance of PAS reactive pyriform cells in successive maturing stages and the appearance of PAS reactive zona pellucida, which was thin in early stages and increased in thickness with the development of PAS reactive follicular pyriform cells is significant. The similar chemical nature of mucosubstances in the pyriform cells and zona pellucida; the appearance of these chemical components, simultaneously in the monomorphic PAS reactive cells, PAS reactive pyriform cells in follicular wall and the concurrent development of PAS reactive zona pellucida, and the evident presence of PAS reactive protoplasmic elongations towards the oocyte membrane, where PAS reactive zona pellucida was being laid; are the suggestive processes indicating the role of the pyriform follicular cells in the formation of PAS reactive zona pellucida.

This type of involvement of follicular cells in the formation of zona pellucida has been studied ultramicroscopically in other reptilian, amphibian and fish oocytes (Blane, 1971, Hubert, 1971 b; Neaves, 1971; Rahil and Narbaitz, 1973); where electron dense zona material is laid down by the follicular cells in between follicular cells and oocyte membrane.

The presence of neutral, carboxy and sulfomucins was observed in the peripheral ooplasm in addition to glycogen in O_3 , O_4 , O_5 and O_6 oocytes. The peripheral ooplasm is the nearest part of ooplasm to the follicular wall; only oocyte membrane, in young oocytes a nd the zona pellucida in vitellogenic oocytes keep them apart. In H.flaviviridis the confluency of the follicular cells with the ooplasm is achieved through the protoplasmic prolongations of pyriform cells (Varma, 1970 b; Guraya and Varma, 1976) and also in other lizards (Gerrard, et al, 1973).

The chemical composition of the mucins studied in pyriform cells and peripheral ooplasm showed presence of glycogen and neutral mucins in common. In H.flaviviridss the confluency of the pyriform cells is achieved through the cytoplasmic elongations with the ooplasm and these prolongations also share the similar mucins and glycogen with the pyriform cells and peripheral ooplasm, possibly indicating the probable transport of glycogen and neutral mucins to the maturing oocytes. The transport of metabolities including lipids, lipoproteins, glycogen, ribonueleoproteins from follicular cells to maturing oocytes in various lizards has been speculated by the number of authors (Bou-Resli, 1974; Ghiara et al. 1968, 1970; Hubert, 1971b, 1973 b, 1976; Neaves, 1971, Rahil and Narbaitz 1973, Taddei 1972 a, Guraya 1978), also stressed the role of follicular cells in the transport of nutrients to the maturing oocytes in lizards.

The cortical region of oocytes became evident in O_6-oocytes. The number of staining methods used for the muco-polysaccharides showed the cortical region of ooplasm possess the carboxy mucins, neutral mucins and sulformucins. The intensity of staining reactions showed poor amount of the three types of mucins reported. In the successive stages of development the amount of these mucins appeared to be increased as judged by the increasing intensity of staining reactions. The cortical region was significantly noted in O_8-oocytes; where the granules showing intense PAS reactivity, intense alcianophilia at AB pH 1 and AB pH 2.5 was noted. By the other techniques employed it was infered that these granules contain neutral mucins, carboxy mucins and sulfomucins. The presence of these granules in cortical region indicates that they are cortical granules which were also detected in the eggs of number of non-mammalian vertebrates. (Arndt 1960; Faratade and Nalawade, 1980, Kille, 1960, Motomura 1952; Osani 1960; Wartenberg 1962; Wartenberg and Schmidt, 1961). The cortical granular nature of these granules was also confirmed by the presence of lysosmal enzymes (Acid phosphatase and β-glucuronidase) in the simultaneous preparations of the H. flaviviridis ovaries. The cortical granules observed in O_8-oocytes also showed neutral mucins, carboxy mucins and sulfomucins in them. Similar type of results were also obtained by Faratade and Nalawade (1980) in Calotes Versicolor oocytes.

The presence of cortical granules in the oocytes is a significant feature of non-mammalian oocytes. Katagiri (1959) and Kemp and Istock (1963, 1967) reported that the cortical granules erput after fertilization and exclude their contents into the perivitelline space.

The stage I atretic follicles showed poor amount of neutral mucins and glycogen in follicular cells and zona pellucida. The other invaded granulosa cells which showed phagocytotic mode (Their phagocytotic nature was confirmed by presence of lysosomal enzymes, acid phosphatase, β-glucuronidase in simultaneous preparation), showed high amount of neutral mucins, corboxy mucins and sulfomucins. The mucopolysaccharides in the different atretic follicles seem to be insignificant as all the atretic follicles are degenerating follicles.

The above discussed results indicate the probable role of mucopolysaccharides in the maturation of oocytes, especially formation of zona pellucida and cortical granules. Their studies in different maturing oocytes of H. flaviviridis also gave probable mode of functioning of follicular cells during maturation of oocytes.

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FIGURE CAPTIONS

Fig 1 - O_1 and O_2 oocytes stained for PAS X 156

Fig 2 - An intermediate stage in between O_2 and O_3 oocytes stained for PAS. F-follicular wall, Arrow - PAS reactive cell X 240

Fig 3 - An intermediate stage stained for AB 2.5 Arrow-peripheral alcianophilia F - follicular wall X 300

Fig 4 - O_3 oocyte stained for PAS. F - multilayered monomorphic follicular wall. Z - thin zona pellucida X 500

Fig 5 - Magnified view of monomorphic follicular wall of O_3 oocyte. Arrow - PAS reactive cell. X 600

Fig 6 - O_4 oocyte stained for PAS g - granulosa layer; F - follicular wall. Z - zona pellucida; Arrow indicates pyriform cells. X 250

Fig 7 - O_4 oocyte stained for AB 2.5. The arrow indicates peripheral alcinophilia. X 600.

Fig 8 - O_5 oocyte with maximum number of pyriform cells in follicular wall. Stained for PAS. G - granulosa cells; F - follicular wall. Z- zona pellucida N - nucleus. X 500

Fig 9 - Enlarged view of follicular wall of PAS stained preparation showing pyriform cells. Arrows indicat protoplasmic prolongations showing confluency with ooplasm X 1300

Fig 10 - O_6-oocyte stained for PAS. G - granulosa cells; F - follicular wall; Z - zona pellucida (PAS reactive and PAS unreactive) C - cortical region. Arrows indicate peripheral PAS reactive ooplasm. X 350

Fig 11 - Advanced stage of O_6 stained for AB 2.5; showing alcianophilia in cortical region. C - cortex region. X 600

Fig 12 - O_7-oocytes stained for PAS. Z - zona pellucida; F - follicular wall; C - cortex region. X 250

Fig 13 - O_7-oocytes stained for AF. The AF-reactivity as observed in cortex region. C - cortex, F - follicular wall. X 180

Fig. 14 - Advanced stage of O_7 oocyte stained for AB pH 1. Cortex region shows alcianophilia. F - follicular wall, C - cortical region. X 150

Fig 15 - Advanced stage of O_7 oocyte stained for AF - AB pH 2.5 Granular reactivity is visible in cortex region (CG); F - follicular wall. X 300

Fig 16 - O_8-oocyte stained for PAS. Z - zona pellucida; G-granulosa cells; F - follicular wall, CG - cortical granules. X 240

Fig 17 - O_9-oocyte - mature oocyte stained for PAS. CG - cortical granules; F - follicular cells yolk globules are evident in inner ooplasm. X 60.

Fig 18 - Atretic follicle stage I stained for PAS. Vacuolar cytoplasm can be noted X 20.44

Fig 19 - Polymorphic follicular atretic follicle stage-I Arrow shows vacuolated follicular wall. X 48

Fig 20 - Monomorphic multilayered follicular walled atretic follicle. Arrow indicates degenerating follicular wall. X 80

Fig 21 - Atretic follicle stage I stained for PAS. Arrow indicates degenerating follicular wall. X 150

Fig 22 - Atretic follicle stage I. Stained for AB pH 2.5. Degenerating follicular wall is evident. X 180

Fig 23 - Atretic follicle stage II stained for PAS. G - granulosa cells. F - follicular wall. Arrow indicates invading follicular cells. X 112

Fig 24 - Atretic follicle stage III stained for PAS - AB pH 2.5 sequence. Arrows indicate invaded phagocytotic cells in follicle . F - follicular wall. X 90.2

Fig 25 - Atretic follicle advanced stage III stained for PAS. Arrows indicate phagocytotic cells. F - follicular wall. X 150.

Fig 26 - Atretic follicle stage IV. Stained for PAS. Arrow shows thick connective tissue. X 57.6

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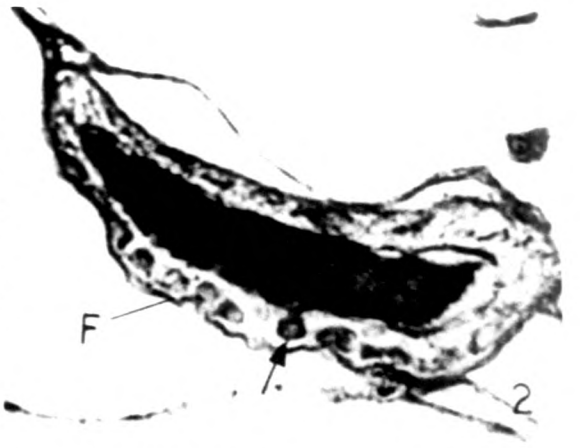

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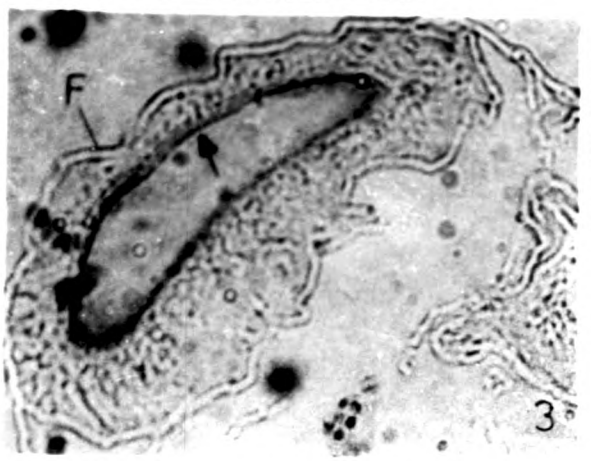

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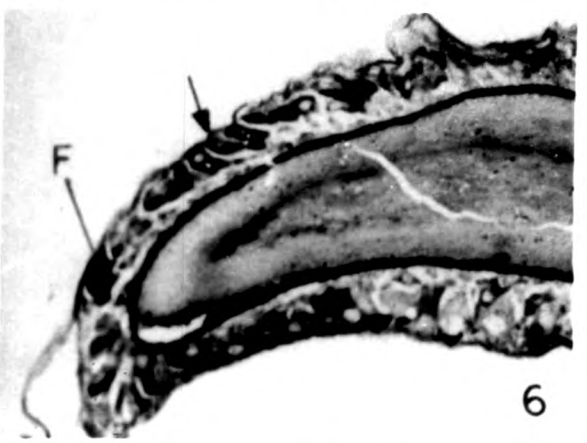

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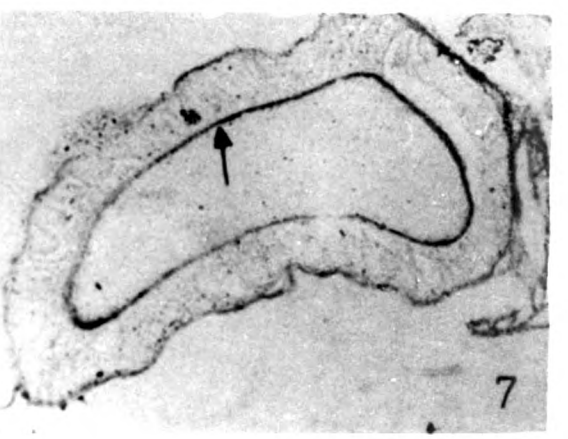
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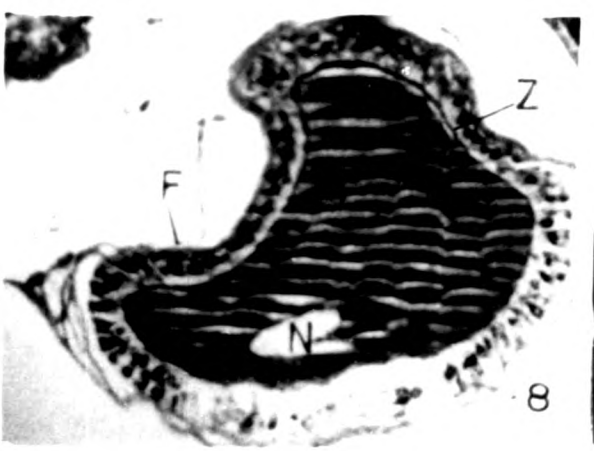

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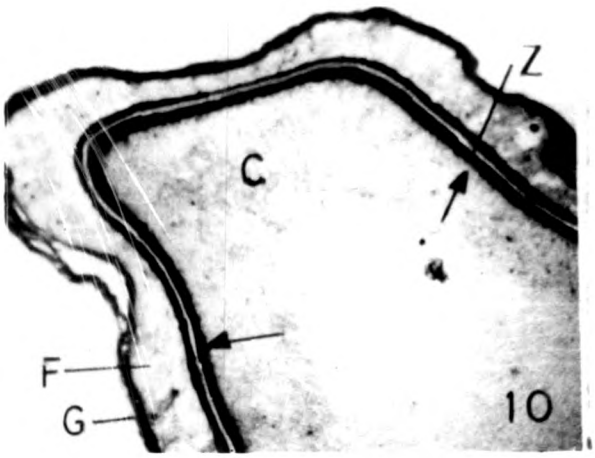
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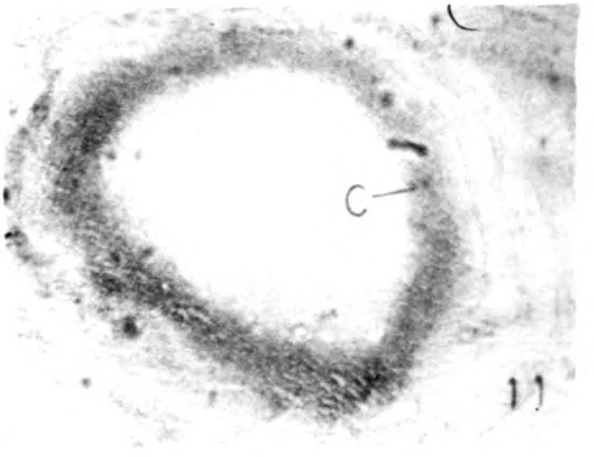
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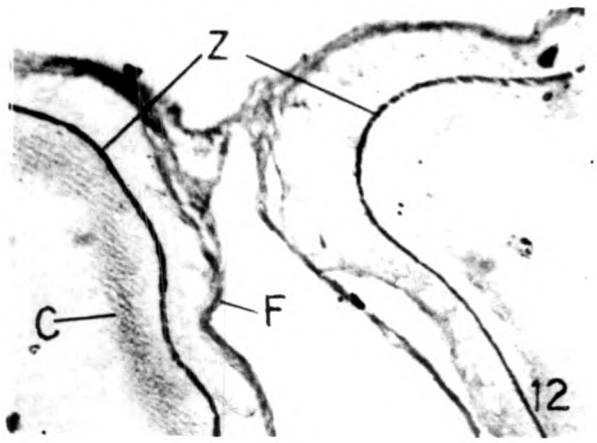
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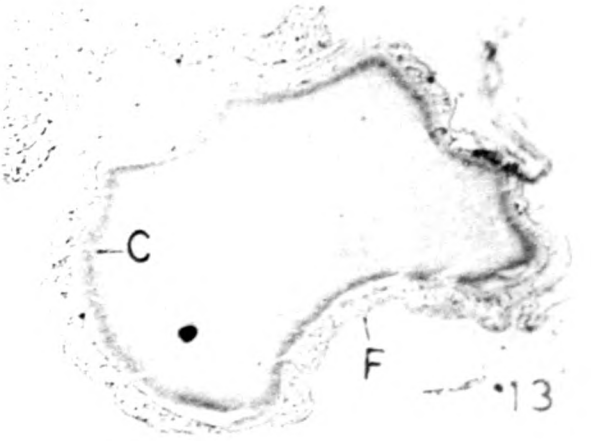
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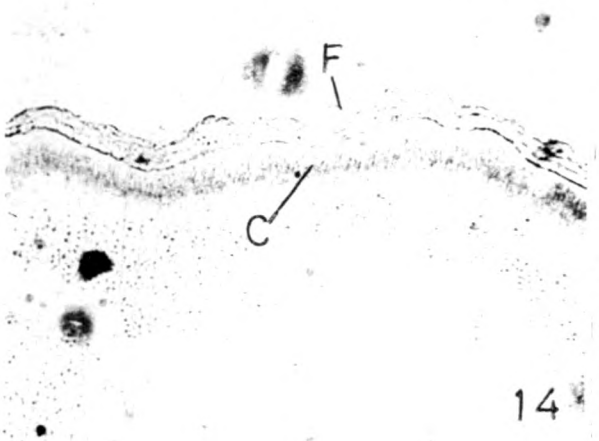
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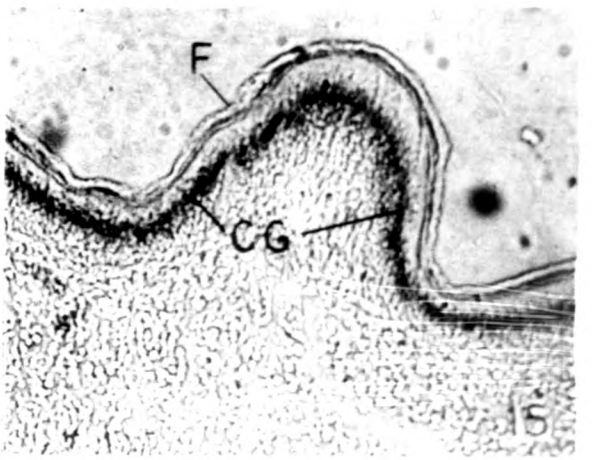
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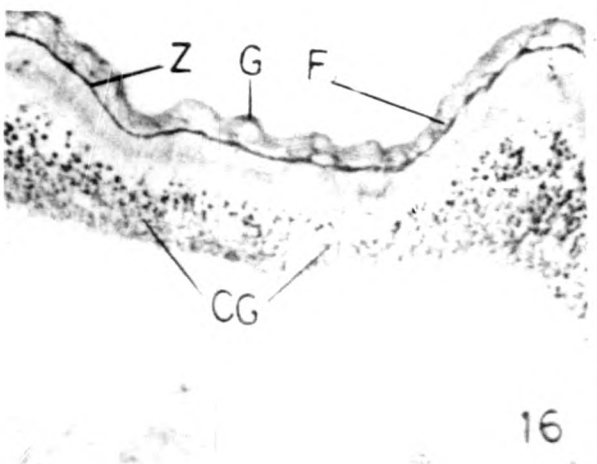
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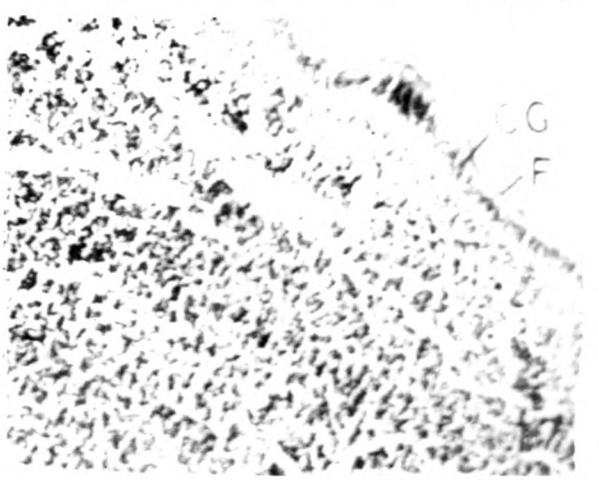
CG
16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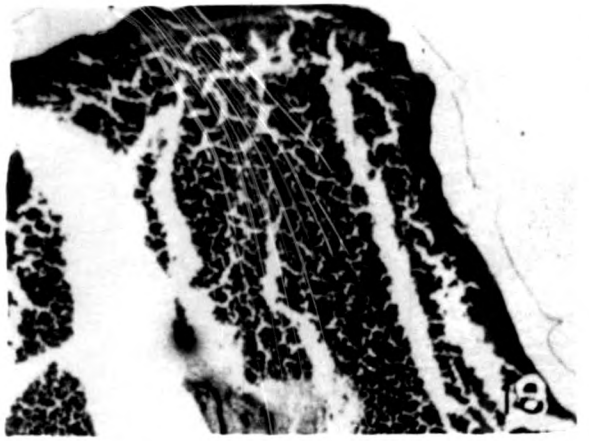

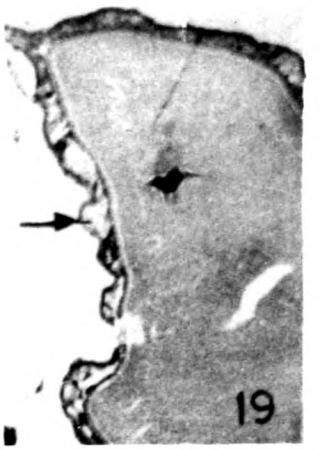

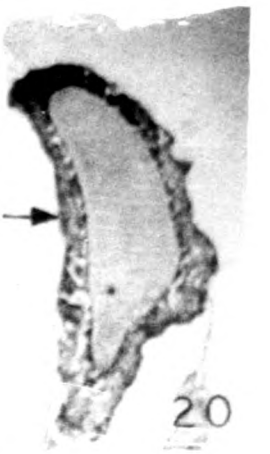

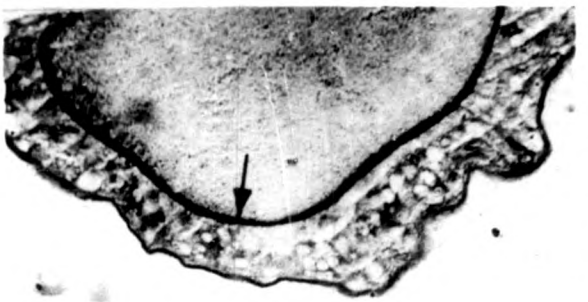

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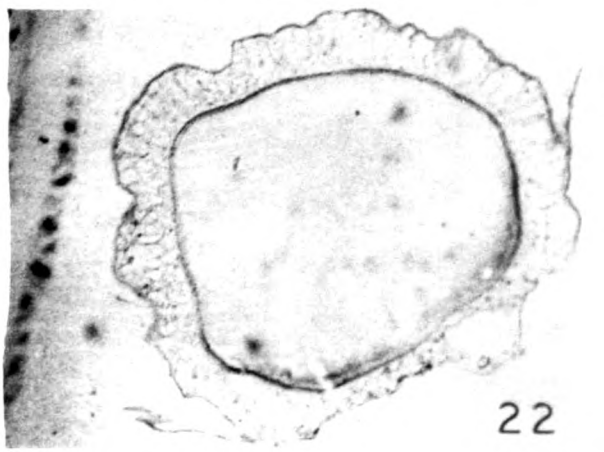

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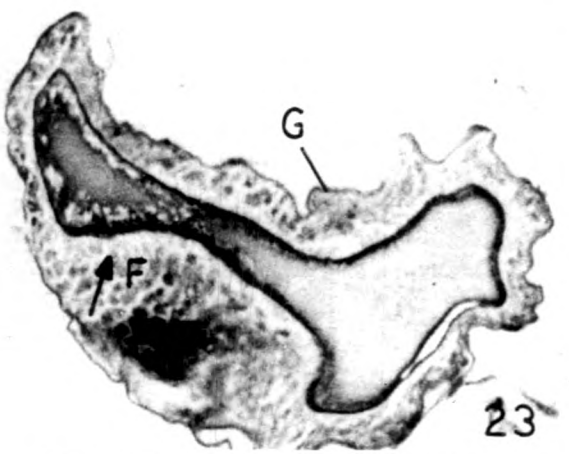

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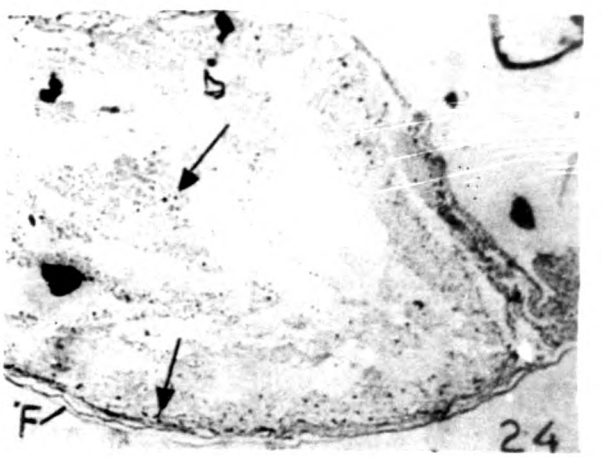

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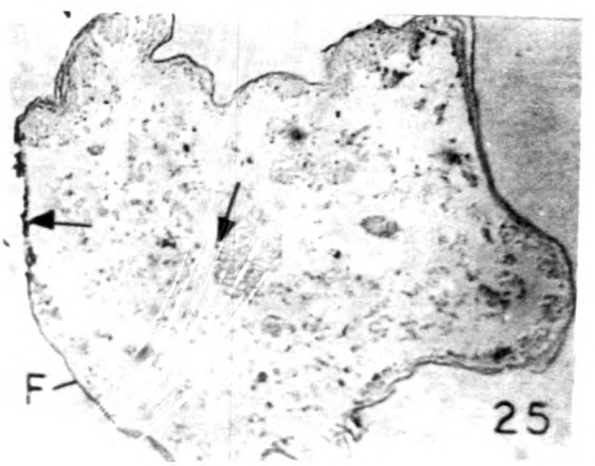

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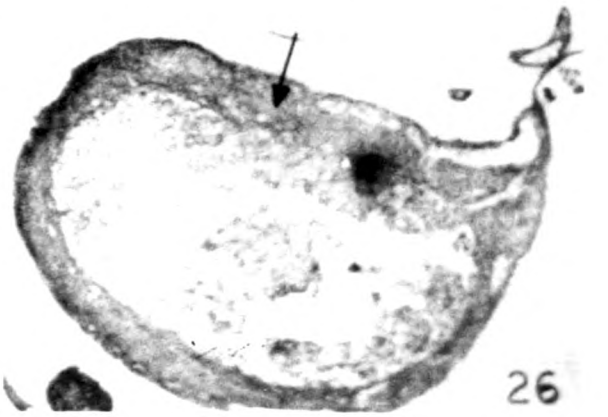

26