THE INTERSTITIAL CELLS IN THE TESTIS OF
ICHTHYOPHIS GLUTINOSUS LINN.

BY B. R. SESHACHAR
(Department of Zoology, Central College, Bangalore)

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NOTHING is known of the structure of, or the seasonal changes in, the interstitial tissue of Apoda and it is the purpose of this paper to describe in some detail the results of my study of this tissue in the testis of Ichthyophis glutinosus. For my work on the spermatogenesis of this animal I have been able to collect specimens throughout the year and am therefore able to present an idea of the changes occurring in the interstitial cells during the different seasons of the year.

Our knowledge of the interstitial tissue of the male Amphibia is itself meagre. Among the urodeles, the study of Humphrey (1921), and of Kolmer and Koppanyi (1923) are important. Champy (1913) and Aron (1921 a, 1921 b) have also described the changes occurring in the interstitial tissue and have discussed at some length the bearing of this tissue on the development of secondary sex characters. In some urodeles like Molge cristata (Aron, 1921) and Pleurodeles waltli (Kolmer and Koppanyi, 1923) this tissue becomes separated from the testis and lies beside it in the form of a distinct secondary body, which Aron found (in Molge cristata) closely related with the development of secondary sex characters. Among the Anura, the work of Friedmann (1898), Mazetti (1911) and Champy (1913) are important. Clearly, so far as the Amphibia are concerned, two definite schools of thought have come to being: (1) the first one including Friedmann (1898), Mazetti (1911), Aron (1921) and Kolmer and Koppanyi (1923) which believes that the development of interstitial tissue is parallel with the development of sex cells, and (2) secondly, the school which includes Champy (1913) and Humphrey (1921) arguing that the two have no relation with each other and that indeed, in some cases (Rana esculenta) the development of the two is never parallel.

This problem is closely related with the functional significance of interstitial tissue. Two functions have been assigned to it. First, the so-called 'trophic' function, which means that the interstitial cells prepare a material...
which is necessary for the sex cells. The second and the more important function attributed to the interstitial cells is the function of the elaboration of hormones whereby they are believed to develop an internal secretion, which, among other things, marks the development of secondary sexual characters. The literature on this subject is extensive and so far as the Amphibia are concerned, the discussion between Champy and Aron has shown that there are really two sides to this problem.

From these points of view the case of *Ichthyophis* is very interesting. I have already reported (1936, 1937) that two definite phases in the activity of the testis can be recognised in this animal. From March till November, the testis is active and spermatogenesis is in full progress. During the winter months, the testis is at rest and the locules are empty. No stages of spermatogenesis are seen. A study of the interstitial tissue reveals that during the months when spermatogenesis is actively proceeding, the tissue is meagre, while during the winter months it increases in volume and is more conspicuous. I have assured myself regarding the inverse development of interstitial and sex cells and believe that the two are negatively correlated in this manner.

Figures 1 and 2 illustrate the two conditions. In the active testis, the interstitial tissue occupies the triangular areas between the roughly hexagonal locules and also similar areas in the periphery of the testis. The septum between the locules is a thin membranous partition with elongated stromal cells and practically no interstitial cells, which are confined almost exclusively to the cornets of the locule. The locules are therefore largest in this condition and are filled with cells in different stages of spermatogenesis. Just the opposite kind of picture is offered by the testis in winter. Fig. 2 illustrates a part of the section of the winter testis. The locules are smaller, having shrunk in size. The septa between the locules are much thicker and are seen to be filled with interstitial tissue. The sex cells are few or are totally wanting and the whole testis presents the picture of a resting condition. The interstitial cells are grouped together in the form of distinct nests separated from one another by connective tissue and numerous such nests of interstitial tissue are seen throughout the testis between the locules. The contrast between the active and resting testis in regard to the quantity of interstitial tissue is very clear and striking.

*Ichthyophis* therefore presents a condition similar to that described by Champy (1913) in *Rana esculenta* where the development of the sex cells and interstitial tissue is never parallel and really takes place at different periods in the year.
Much has been written about the significance of interstitial tissue in the development of secondary sex characters, and even in Amphibia, two opinions obtain regarding this aspect of the problem. Champy (1913) has been unable to establish any relation between the two, while Aron (1921) and others clearly see a connection between the two. The case of *Ichthyophis* is very interesting. In this animal and in Apoda generally, external indications of sex, even during the breeding season, are wanting, and further, if the interstitial tissue is to be correlated with the development of external sex features, these characters must evidently be developed in winter when the interstitial tissue is at its highest and most abundant development. The absence of the development of any apparent secondary sexual characters at any period in the life of the animal goes to show that the interstitial tissue is not necessarily correlated with the development of secondary sexual characters.

It is now an established fact that in the majority of animals, the quantity of interstitial tissue varies in different seasons of the year. But it is a far more difficult matter to account for this variation in the tissue, and the exact manner in which the increase in the tissue at certain seasons of the year is brought about is not clear. From an examination of the literature on the subject it would appear that the increase in interstitial tissue is due to one or both factors: (1) Cell multiplication, involving the division, either by the amitotic or mitotic method, of the existing cells, thereby increasing their number. Direct amitotic division has been reported in interstitial cells by von Bardeleben (1897) in *Homo*, and must be considered, even when present, as a very rare and probably exceptional phenomenon. Division of interstitial cells by mitosis is certainly more common and there is no doubt that some contribution to the increased number is made in this manner.
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[Von Hansemann (1896); Rienke (1896); Von Lenhossek (1897)] in the human species. (2) A second method of increase in interstitial tissue has also been reported by a number of workers and takes the form of a transformation of stromal cells of the ordinary connective tissue type into typical interstitial cells [Mazetti (1911) in Rana; Rasmussen (1917) in Marmota; Humphrey (1921) in Necturus and other urodeles].

In this connection a paper by Blount (1929) is of considerable interest. Working on the horned toad (Phrynosoma solare) he has determined the seasonal changes occurring in the interstitial tissue and their correlation with the structure of the testis. His conclusions are interesting. He draws a significant distinction between the volume of interstitial tissue and the number of interstitial cells. He finds the volume of the tissue greatest during the breeding season. The size of the individual cells of the tissue is also very large at this period. But soon after the breeding season, the volume of the tissue decreases while the number of the cells is increased. How this is done is not clear. Apart from the fact that the author has drawn a distinction between the volume of the interstitial tissue and the number of cells of the tissue, the problem of the increase in the number of cells (which, in this animal, takes place after breeding) remains the same. If the number of cells is minimal during the breeding season, the question will be asked, how is the number reduced during the breeding season? Blount does not subscribe to the view of an inter-transformation between interstitial and stromal cells and does not notice any evidences of such a change in his material.

Now, with reference to Ichthyophis the problem of the seasonal changes in the interstitial tissue may be examined. It has already been observed that two distinct conditions can be noticed in regard to the interstitial tissue in this amphibian and that the development of the sex cells and of interstitial tissue appears to be correlated in a negative manner. I have not been able to make any tests of the type Blount has made in Phrynosoma and must content myself with changes as evidenced by appearance and quantitative examination.

It must be mentioned here that none of the authors who have seen either amitotic or mitotic divisions in the interstitial tissue of the animals they have studied is quite convinced regarding these divisions constituting the sole method of increase of the interstitial tissue. Divisions of either kind are too seldom to be of any value in increasing the tissue. The authors themselves admit this fact. Further, conflicting evidences regarding the occurrence or otherwise of these divisions in the same animal as examined
by different investigators add to our difficulty in accepting this method of increase of the tissue.

In *Ichthyophis*, I have not seen enough evidence for the conclusion that either mitotic or amitotic divisions contribute to the increase of interstitial tissue. I have not observed direct division in the nuclei of these cells, nor have I seen bi- or multinucleate cells at any time. A few mitotic figures have been encountered (Fig. 3) but never in such numbers as to warrant the conclusion that they are responsible for the increase of the tissue. These rare mitotic figures do not occur with any regularity. They are found at all times of the year and I believe they have no significance in the increase of the interstitial tissue in *Ichthyophis*.

The second method of the increase of this tissue remains to be studied. Rasmussen (1917) and Humphrey (1921) are amongst those who have observed a periodic transformation of stromal cells into interstitial cells and later, a regression into stromal cells. The increase and decrease of tissue (which, in *Ichthyophis*, means also an increase and decrease in the number of cells) are therefore due, according to them, to this transformation and retransformation. It means that at the season when the interstitial tissue is at its maximum, few stromal cells are seen and that when the interstitial tissue is poorly developed, the stromal cells are large in number. This is not borne out by the conditions found in *Ichthyophis*. No such inverse correlation between stromal and interstitial cells can be established. On the other hand, it is noticed that in the winter testis, correlated with the large quantity of interstitial tissue, large numbers of stromal cells also are found. I have no doubt that the stromal cells, like the interstitial cells, are larger in number and quantity in winter than during the rest of the year and that in fact, the increase in quantity applies as much to the stromal tissue as to the interstitial tissue.

One point of interest remains to be noted here. The disposition of interstitial cells in winter differs from that during the rest of the year. During the months when spermatogenesis is in progress in the testis, the cells occur as heaps in the triangular areas between the locules (Fig. 4). In the winter testis on the other hand, the cells occur in the form of groups or nests and each nest appears to be bound by a common membrane (Figs. 5 and 6). Isolated cells, either singly or in very small numbers do occur, but there is, on the whole, an orderliness in the grouping of the interstitial cells of the winter testis which is absent in the active condition. A similar arrangement has been noticed by Rasmussen (1917) in *Marmota*. He found this kind of grouping only in animals and in seasons where the interstitial
tissue showed maximum development, a finding which corresponds with mine in *Ichthyophis*, where in the winter testis, with the maximum development of interstitial tissue, this kind of grouping is seen. Rasmussen says "this would suggest that there is cell division and that each group of cells represents the daughter cells of a single parent cell". But he has found no direct evidence of either mitotic or amitotic cell division, though occasional bi-nucleate cells are seen. My position is very similar to that of Rasmussen. While so far as appearance is concerned, the aforesaid grouping of cells suggests cell division, the actual evidence is insufficient to corroborate it and I also am obliged to leave the problem in this inconclusive condition.

**Structure**

In the active as well as resting conditions of the testis, the interstitial cells are the most conspicuous secondary cells of the organ. In the active testis they occur as isolated groups in the interstices between the locules or in the periphery and are far fewer in number than in the resting testis, an observation which is in conformity with that of Blount (1929) in *Phrynosoma*. The large size of the swollen capsules leaves very small triangular areas at the corners in which the interstitial cells are found. Very rarely do we find interstitial cells along the walls separating the adjacent locules. These walls are very thin and contain few cell elements of which the stromal cells are the most conspicuous. In regard to the size of the cells at the different seasons of the year, my observations confirm those of Blount. During seasons of greatest spermatogenetic activity the size of the individual interstitial cell is much larger than that during the season when the testis is at rest. In *Phrynosoma solare* Blount has also found that during the breeding season the greatest size in the interstitial cell is reached. In *Ichthyophis*, the size of the cells varies from 24 microns in the resting testis to 48 microns in the active testis.

The size of the nucleus, however, in the two conditions is apparently unvariable and I believe no difference in the size of the nucleus can be detected in the two seasons. Bi- and multi-nucleate cells observed in certain animals [Winiwarter (1912) in Man; Rasmussen (1917) in *Marmota*; Duesberg (1918) in the opossum] are not found in *Ichthyophis*. The extensive studies of Humphrey (1921) in a number of urodeles have not revealed any such cells in those Amphibia.

The nucleus of the interstitial cell is usually a deeply staining body. It is loaded with chromatin and stains far more intensely than the germ cell elements of the testis. Often it exhibits a definite indentation at one pole, generally at the pole in the neighbourhood of the centrosome. In
tissue fixed in Mann-Kopsch and Kolatschew fluids it is seen to be irregular in shape, evidently due to distortion. A number of nucleoli are present.

In the neighbourhood of the nucleus,—and if the nucleus is cup or kidney-shaped, in or near the concavity,—is the centrosome (Fig. 7). In Bouin preparations it assumes the form of a dense mass of protoplasm. In the centre of this dense cloud are the two centrioles which are always in the form of granules. Distinct radiations are seen in the centrosomal plasm.

In material fixed in osmic fluids, more particularly in Mann-Kopsch and Kolatschew material, the Golgi bodies are seen to occupy this region. They are in the form of a variable number of irregular curved rods of various sizes concentrated in the region of the centrosome (Fig. 8). A distinct Golgi region can therefore be distinguished in the neighbourhood of the nucleus in all preparations treated for Golgi bodies. Duesberg (1918) is probably the first to describe the Golgi apparatus in the interstitial cell but to my knowledge it has never before been described in the Amphibian interstitial cell. In his exhaustive and critical study of the interstitial tissue in the urodeles Humphrey (1921) makes no mention of this apparatus. I believe that the Golgi apparatus in the interstitial cell of *Ichthyophis* is in the form of a tangled network as depicted by Duesberg (1918) in the opossum.

Mitochondria in interstitial cells have long been known and have been described by a number of workers. Jordan (1911) was probably the first to study them. Winiwarter (1912) found them in Man and particular attention has been paid to them by Duesberg (1918) in the opossum and by Rasmussen (1918) in *Marmota*. So far as the Amphibia are concerned, the only work which makes mention of mitochondria in interstitial cells is that of Humphrey (1921) who has dealt with them at some length. In *Ichthyophis* they are quite conspicuous and are in the form of both grains and short rods. They may also occur as chainlike formations by fusion with one another, a very common feature. They lie scattered in the cytoplasm.

The presence of large quantities of lipoid material in the cytoplasm characterises the interstitial cells. Exceptions have so far been reported from the wild boar (Plato, 1896) and the Pig (Whitehead, 1908). Their presence has also been denied in the opossum by Jordan (1911) but the later studies of Duesberg (1918) on this animal seem to indicate that the lipoid content of interstitial cells tends to vary considerably, not only in different species but also in the same individual. The lipoid globules are of varying sizes in *Ichthyophis* and in a fully developed state they almost completely fill the cytoplasm, so that in osmic fixatives the whole cell looks quite black. In such cells the other cytoplasmic bodies and even the nucleus appear difficult to be
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Discerned. If the slides are treated with turpentine most of the fat globules become decolourised and the contents of the cell become clear. Such a cell is shown in Fig. 9. It is seen that the whole cytoplasm presents a bubbly appearance indicating the presence of fat globules practically crowding the cell space. The nucleus is clear and the Golgi bodies are seen at one pole of the nucleus in the form of a number of rods crowded together.

An examination of a cell where fat is being deposited reveals an interesting picture. In such a cell figured in 10, all the three cytoplasmic constituents, fat, Golgi bodies and mitochondria can be clearly seen. The Golgi bodies in the form of large irregular rods are conspicuous in the neighbourhood of the nucleus. The fat globules are mainly confined to this region and the mitochondria are scattered in the cytoplasm. The fat globules appear to arise as a result of the activity of the Golgi bodies. The origin of fat in the interstitial cell has not been accounted for by any worker satisfactorily. More than one kind of secretory material has been described but a satisfactory account of the origin of any of them has not been given. From my examination of the interstitial cells of the testis of Ichthyophis I am inclined to believe that the lipoid material which appears to be the only kind of secretion found in the interstitial tissue of this animal takes its origin as a result of the activity of the Golgi bodies.

From an examination of the cytoplasm of the interstitial cell it would appear that the deposition of fat in the cell is not in the nature of a transformation of the Golgi bodies into fat globules. For, even in the cell which is literally packed with fat globules, the Golgi bodies occur as rods in the neighbourhood of the nucleus (Fig. 9), crowded and pressed against the nuclear wall. This is only to be expected from the nature of things. The interstitial tissue is a permanent tissue of the testis and though its precise role is still in dispute, it is to be expected that it is of permanent usefulness to the animal. I believe that the deposition of fat is a continuous process in the interstitial cell, lasting over a long period, perhaps throughout the life of the animal. As such it is to be expected that the Golgi apparatus can only play the part of a structure that deposits fat, itself undergoing no change in the process. Secretion material other than fat has been reported by a number of workers, either intracellularly or intercellularly. Whitehead (1908) has seen them in a variety of animals. Rasmussen (1917) finds them in Marmota. Duesberg (1918) reports in the opossum networks inside the cell which he considers are in the nature of secretion products and which he believes are due to the transformation of mitochondria. This author finds similar material in the intercellular spaces and appears to think that this offers evidence of the secretory material finding its way into the circulation.
Humphrey (1921) has seen secretions in the interstitial cells of urodeles and believes that they are allied to and only slightly different from mitochondria.

The interstitial cells of the winter testis offer pictures which in many respects are different from those of the active testis. It has already been remarked that the construction of the interstitial tissue in the two conditions of the testis is different but the minute structure of the cells also is different. An interstitial cell from the winter testis is shown in Fig. 11. The nucleus is vesicular and the chromatin appears to be restricted to a few large bodies suspended in the vesicular nucleus. The cytoplasm reveals two distinct structures. First there is a cloud of granules which are usually grouped together in one place and at one pole of the nucleus. These granules are larger than the mitochondria encountered in the typical interstitial cell of the active testis and while they tend to group themselves in one place in the cell, a few may lie scattered in the cytoplasm. The second constituent of the cell takes the form of an amorphous lake occupying a large space in the cell. More than one such lake may be found in the cytoplasm of a cell. Both these structures take a deep black stain in haematoxylin, but while the former remain distinct and clearly separate from each other, the latter is a large dark body. It was at first thought that this different appearance of the interstitial cell of the winter testis was due to faulty fixation but examination of a number of sections prepared in a variety of methods corroborated the description of the cell given above. This was also confirmed by the appearance of the cell in Mann-Kopsch material where both the lake as well as the granules go black in osmic acid.

I have been unable conclusively to determine the real nature of these two bodies. Whitehead (1908), Rasmussen (1917), Duesberg (1917) and Humphrey (1921) have noticed in their material bodies larger than and different from mitochondria, but which are probably modified or distorted mitochondria. And it is possible that the laky material found in Ichthyo-phis might correspond to the amorphous secretion found by Duesberg in and outside the interstitial cells of the opossum. But I have not found intercellularly any secretion material corresponding with what Duesberg (1917) and Wagner (1923) have seen and am therefore unable to say what this material can be.

Summary

The interstitial tissue of the testis of Ichthyophis glutinosus varies in quantity and distribution in the active and resting conditions of the testis. In the former it is in the form of scattered groups of cells in the interstices of the locules, while in the latter it occurs as large number of nests of
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cells between the locules. Fat is a characteristic cytoplasmic content of the interstitial cell and is believed to have arisen by the activity of the Golgi bodies. The significance of this fat or the general functional significance of the interstitial tissue of the testis is unknown.

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EXPLANATION OF FIGURES

Fig. 1. A part of a longitudinal section of *Ichthyophis glutinosus* during spermatogenesis. The locules are large and are separated by thin septa. Interstitial cells are found at the corners. × 75.

Fig. 2. A part of a longitudinal section of the testis of *Ichthyophis glutinosus* in winter when spermatogenesis is at rest. The septa are thicker and are filled with nests of interstitial cells. × 75.

Fig. 3. An interstitial cell in mitosis. Such mitoses are rare in *Ichthyophis glutinosus* and are considered of no value in the increase of the tissue. × 400.

Fig. 4. A corner of a locule of the active testis showing the disposition of interstitial tissue × 200.

Fig. 5. A portion of the interstitial tissue in the winter testis. The nest-like disposition of cells is clearly seen. × 200.

Fig. 6. A single nest of the same magnified. × 800.

Fig. 7. An interstitial cell showing the centrosome and the centrioles. Bouin. × 1800.

Fig. 8. The same: a Mann-Kopsch preparation showing the Golgi bodies. The vacuoles indicate the position of the lipoid globules which have been dissolved out. × 1800.

Fig. 9. An interstitial cell showing great vacuolation of its cytoplasm. The Golgi bodies are pressed close to the nucleus. Mann-Kopsch. × 1800.

Fig. 10. An interstitial cell showing the process of deposition of lipoid material. Its relation with the Golgi bodies is obvious. The small irregular rods and granules are the mitochondria. Mann-Kopsch. × 800.

Fig. 11. An interstitial cell of the winter testis. The cytoplasm shows a number of granules and deeply staining masses of material. See text. Flemming without acetic. × 2400.

Fig. 12. Photomicrograph of a group of interstitial cells in active testis. × 375.

Fig. 13. A portion of the interstitial tissue of the winter testis showing the nest-like arrangement of cells. × 130.

Fig. 14. A Mann-Kopsch preparation of the interstitial tissue of active testis showing the vacuolated cytoplasm and the Golgi areas. × 375.

Fig. 15. A single interstitial cell showing the vacuolated cytoplasm, nucleus and the Golgi apparatus adjacent to the nucleus. Mann-Kopsch. × 550.

*D. Collecting Duct; G. Golgi Apparatus; G.C. Germ Cells in various stages of spermatogenesis; Int. C. Interstitial Cells; N. Nucleus.*