STAGES IN THE SPERMATOGENESIS OF SIPHONOPS ANNULATUS MIKAN. AND DERMOPHIS GREGORII BLGR. (AMPHIBIA : APODA)

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For some years past the author's interest has been aroused by the peculiarities in the gametogenesis of the members of the Apoda and has found expression in a number of papers on the various aspects of its study. India is the home only of four genera of Apoda of which two are very rare. The more common genera (Ichthyophis and Urceotyphlus) which have been studied by the author have given indications of the importance of the study which has tempted him to continue his investigations and extend them to the other genera of Apoda. The present paper is the result of this effort and marks an attempt to consolidate our available knowledge of the spermatogenesis of this interesting group. In this the author has not been as successful as he would have wished to have been. The material which forms the subject-matter of this paper is not Indian and evidently had not been treated for the study of the chromosomes. The chromosomes, their number and behaviour,—have formed a very important part of my earlier studies on the spermatogenesis of Ichthyophis and Urceotyphlus and it is therefore with considerable regret that I state that I am unable to write on this aspect of the question. But feeling that it might be a very long time before fresh material may come my way, I have thought it fit to describe the general process of spermatogenesis in Siphonops annulatus and Dermophis gregorii and compare it with that in Ichthyophis glutinosus and Urceotyphlus narayani.

A single male specimen of each of these two species came into my possession. For the specimen of Siphonops annulatus I am grateful to Prof. A. Subba Rau who obtained it in turn from Prof. J. P. Hill's collection in the University College, London. The specimen of Dermophis gregorii is the property of Dr. L. S. Ramaswami who kindly allowed me to examine the form and remove the testis. I am grateful to both Prof. A. Subba Rau and Dr. L. S. Ramaswami for placing at my disposal this valuable material.
The specimen of *Siphonops annulatus* was fixed in corrosive sublimate acetic and later transferred into alcohol. The fixation of *Dermophis gregorii* is not known. Both of them had been lying in alcohol for a long time before they came into my possession but the preservation of the former is very much the better of the two. It has given me all the information I wanted on the spermatogenesis of this species, except the chromosomes. The preservation of *D. gregorii* is not very satisfactory but some of the stages stand out clearly and have lent themselves for a correct and clear interpretation.

I wish to thank Prof. A. Subba Rau for his kindness and encouragement throughout the course of this study and for his many helpful suggestions.

*Structure of the testis.*—All the Apoda agree in the disposition of the testis. It occurs as a number of beaded structures strung together along the collecting duct as has already been observed in *Ichthyophis* and *Uraotyphlus* and as noticed again in *Dermophis* and *Siphonops*. The number of testis lobes, however, is subject to variation, not only in the different species but also in the different individuals of the same species. I found only three lobes on each side in the specimen of *D. gregorii* that I dissected. I cannot say if this is the normal number found in the species. At any rate it represents the lowest number of testis lobes found by me in any Apodan example. The size of the individual testis lobes is also subject to variation and the remarks I made about the significance of this variation in *Ichthyophis* and *Uraotyphlus* apply to the present genera also.

The microscopic structure of the testis of the two genera under examination shows a close similarity with that of *Ichthyophis* and *Uraotyphlus*. The external appearance of each testis lobe shows a number of rounded elevations which have been described by earlier workers (Spengel, 1876; Tonutti, 1931) as resembling a bunch of grapes. Each of these elevations represents a locule and a number of such locules make up the testis. In regard to the size of the locules the genera under examination differ a little, those of *Dermophis* being slightly the larger of the two.

In the matter of the microscopic structure of the testis, the Apoda exhibit two distinctive characters. First, the disposition and size of the locules both show a very distinct and clear departure from either the urodelan plan on the one hand or the anuran plan on the other. The locules are very large in the Apoda as compared with the Urodela or the Anura and are separated by clear thin septa (Fig. 1). Each locule is filled with a fatty matrix in which are embedded cell groups in different stages of spermatogenesis. This matrix is very characteristic of the Apoda and has been found by me
in all the four genera examined and has also been found by Tonutti (1931) in *Hypogeophis*. The presence of this matrix imparts an appearance to the testis which is different from the crowded nature of the cells in the testis of either the Anura or the Urodela. In the latter groups, the comparatively small sized locules and the large number of cells (in different stages of spermatogenesis) give a packed appearance to the entire organ,

which is not seen in the testis of the Apoda at any time of the year or in any period of spermatogenesis. Never at any time have I seen the locules of the testis packed fully with cells as in the other two groups of Amphibia. Even at the height of its activity much of the space in the locule is occupied
by the fatty matrix and it is only in this matrix that the germ cells lie embedded. Indeed, it would appear that this matrix is essential for the development of the cells themselves.

The second point of interest is the uniformity of this testis structure throughout the group Apoda. So far the structure of the testis of five genera is known; four studied by me and one (Hypogeophis) by Tonutti (1931) and in all these the peculiar and unique microscopic appearance of the testis described above, is seen. In this respect, therefore, the Apoda is a close knit homogeneous group.

To summarize: the unique features of the testis of the Apoda consist in (a) the segmented nature of the organ which is resolved into a number of lobes strung along the collecting duct and which extends over a greater part of the length of the animal on either side of the alimentary canal; (b) the large size of the locules of the testis; these locules are not tubular as in the Urodela or the Anura but are more or less spherical and vary in number according to the size of the testis lobe; (c) the peculiar arrangement, in groups, of the sex cells in the locules. Each locule exhibits a number of cell groups each of which is in a certain stage of spermatogenesis and such groups occur scattered and embedded in the fatty matrix that fills the locule. Generally the cell groups in earlier stages of spermatogenesis occur near the periphery of the locule while those in later stages and those undergoing spermateleosis occur nearer the centre of the locule. This arrangement is unique in the Amphibia, in the other two groups of which neither the fatty matrix nor the scattered arrangement of cell groups is found, and where cells in different stages of spermatogenesis are packed together in the available space found in the narrow tubules.

The question may be asked if it is possible to account for this variation in the external and internal structure of the testis of the Apoda. A partial answer may probably be found for the former. The lobed nature of the testis may be a result of the elongation of the body of the animals of this group. Even this is only a partial answer; for, while elongation of the body brings about, on the analogy of other animals, an elongation of the organs and also an asymmetrical development of the organs of the two sides, it does not ordinarily produce a segmentation of the organ. It is probable that the reason for the lobing of the testis in the Apoda is a more fundamental and deep-seated one and must be looked for in the ancestral condition of the group. An answer to this cannot be provided at this stage.

The origin and significance of the second structural feature of the testis is even more obscure. The very large size of the testis locules as compared
with those of the other groups of Amphibia is as inexplicable as the development of the peculiar matrix filling the locules.

The relation between the collecting duct and the locules has been described by me in Ichthyophis and Uræotyphlus where I have shown that the longitudinal collecting duct runs through the testis following the contour of the locules and giving off short side branches to the latter. The locules of Dermophis gregorii resemble those of Ichthyophis and Uræotyphlus in size while those of Siphonops annulatus appear to be much smaller comparatively. Spengel (1876) in his observations on the structure of the testis of Apoda indicates the relation between the testis locules and the collecting duct such that the latter runs in the centre of the testis with the locules arranged radially around it. In Ichthyophis and Uræotyphlus this regular relationship between the two could not be distinguished and it was observed that the collecting duct followed irregularly the interstices of the locules giving off smaller ducts to the locules. I find the same kind of arrangement in Dermophis also. In Siphonops on the other hand, a very slight trace of this central position of the duct and the peripheral position of the locules is seen (Fig.17). The main collecting duct appears to be central, with the locule
arranged peripherally. But it must be mentioned that this arrangement is not constant in *Siphonops* as seen in Fig. 2.

*Spermatogonia.*—My remarks regarding the origin of primary spermatogonia in *Ichthyophis* hold good for the two genera under examination. I have pointed out how in *Ichthyophis* the cells lining the ducts of the testis undergo transformation and so become germ cells. Large numbers of these cells were constantly found at the mouth of the duct in the locule in *Ichthyophis* and *Urceotyphlus*. I believe, in the two genera under examination also the duct mouth forms a constant source of primary spermatogonia in the adult (Fig. 3). Some of these cells at the mouth of the duct become large with conspicuous spherical and polymorphic nuclei while others invest them and become the follicle cells. From the close similarity which the sections of the testis of *Siphonops* and *Dermophis* present to the sections of the testis of *Ichthyophis* and *Urceotyphlus*, I have reasons to believe that the origin and behaviour of the primary spermatogonia is very similar. Arising in this position the primary spermatogonia migrate along the wall
of the locule taking up positions along it (Fig. 19) where they start to divide and grow and pass through meiosis.

The size of the primary spermatogonium is subject to great variation, according to its age and activity. In *Siphonops* it varies from 25 to 40 microns while in *Dermophis* the variation is between 16 and 30 microns.

The problem of polymorphism of the nuclei of primary spermatogonia has been dealt with by me in sufficient detail in *Ichthyophis* (1936) and in *Uraeotyphlus* (1939) and I need not dwell on it here at any great length.

My conclusions regarding polymorphism are amply borne out by my observations on *Siphonops* and *Dermophis*. The spherical form of the nucleus denotes an earlier condition and the polymorphism (Figs. 6 and 20), which is never very pronounced and which is similar to that encountered in other Apoda indicates a condition of particularly heightened metabolic activity. The nucleus however, always reverts to its spherical form just before division (Figs. 7, 8 and 22). The number of nucleoli is subject to great variation and from a condition where there are one or two nucleoli (Fig. 5)
to one where there are several (Figs. 4 and 21), all gradations occur. As in Ichthyophis and Uræotyphlus the staining reactions of the nucleus of the primary spermatogonium also vary according to its spherical or polymorphic condition, being deeper in the former and fainter in the latter.

Of the cytoplasmic bodies I am able to speak only of the centrosome; for this, along with the contained centrioles, is the only object that is at all preserved in the material. Even the centrioles are not always preserved well enough to be seen clearly. This is especially so in regard to Dermophis where the fixation is not as good as in Siphonops. In this latter material, however, the centrioles are quite clear and occupy the centre of the archplasmic area which bears the same relationships with the nucleus and cell in general as in Ichthyophis and Uræotyphlus (Fig. 21). These relationships leave me in no doubt as to the topography of the different cytoplasmic inclusions of the primary spermatogonium, which, had they been well fixed and preserved, would have revealed the same arrangement as in Ichthyophis and Uræotyphlus.

A word about the amitotic divisions of the nucleus of the primary spermatogonium. In my paper on Ichthyophis (1936) I discussed this matter fully and subscribed to the view of Wilson (1928) that amitosis here, as elsewhere, means nothing more than a fragmentation of the nucleus with an attendant increase in the nuclear surface and that in no case could a division significance be attached to it. In Ichthyophis I did find, though extremely rarely, a few isolated instances of binucleate spermatogonia. In Siphonops during my examination of the very limited material at my disposal I found a single primary spermatogonium with two nuclei (Fig. 23). I was unable to determine the nature and condition of the cytosome but I feel that it is unnecessary to deviate from the conclusions drawn in case of Ichthyophis, that amitosis here, as in Ichthyophis, has no division significance and means nothing more than a temporary change involving an increase of nuclear surface.

The onset of division brings about a conversion of the polymorphic nucleus into a regular spherical condition and so far as I can see, the changes that occur in the nucleus and cytoplasm are similar to what I have reported already in Ichthyophis and Uræotyphlus. The nucleus shows blocks of chromatin connected by filamentar processes and these are the forerunners of the definitive chromosomes. A stage of the kind is shown in Fig. 7. The difference between the nucleus in a state of rest and a stage just prior to division can be seen on comparing Figs. 21 and 22. In the former, the nucleus appears granular, the granules filling the cavity of the nucleus more or less evenly, while in the latter, the nucleus appears like a vesicle the chromatin having
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become aggregated into a few coarse masses leaving conspicuous clear spaces in between them. This difference was also observed in the two genera studied earlier.

The primary spermatogonium divides by mitosis and the products of division remain together just beneath the locule septum. The number of divisions a primary spermatogonium passes through before meiosis starts has been a matter of interest to me and I have determined that in *Ichthyophis* the number of divisions is eight, while in *Uraeotyphlus* it is more irregular, the primary spermatogonium passing, sometimes through six divisions and sometimes through seven.

In the present two genera also I tried to count the number of divisions the primary spermatogonium passed through before meiosis set in and so far as the material at my disposal enabled me, and based on the countings of the metaphase plates of the first meiotic division, I found that in *Siphonops* the number of divisions was seven and in *Dermophis* it was slightly irregular, being either six or seven. From an examination of the conditions obtaining in the four genera of Apoda I have so far studied, I am in a position to conclude that the number of divisions a primary spermatogonium in the Apoda passes through before meiosis, though subject to some variation, is six, seven or eight. So far it is only in *Ichthyophis* I have noticed evidences of eight divisions.

Spermatocytes.—After the completion of the divisions by mitosis, the cells, which are now the primary spermatocytes, enter on the meiotic phase. At first, as in *Ichthyophis* and *Uraeotyphlus*, these cells are arranged along the periphery of the locule in two rows (Fig. 24) and form a compact mass. But as meiosis proceeds, the cells leave their peripheral position and migrate inwards into the locule, the individual cells of the same group occurring together but the cell groups themselves being separated from one another and lying in the matrix filling the locule (Figs. 1 and 2).

I have observed both in *Ichthyophis* and *Uraeotyphlus* a stage of rest intercalated between the last division of the spermatogonia and the prophase of meiosis of the spermatocytes. In this condition the nucleus shows a large number of blocks of chromatin. But there is no attendant diminution of basophily of the nucleus as has been observed in a number of Amphibia, notably in *Rana* (Witchi, 1924) and in *Bufo* (Saez and others, 1936). In *Siphonops*, where this stage could be observed with great clearness, it was even more pronounced than either in *Ichthyophis* or in *Uraeotyphlus* (Figs. 9 and 25).
The first stages of meiosis appear to be similar to those described in the two Indian genera. The leptotene bouquet is built up as in *Ichthyophis* and *Uraotyphlus*. The polar orientation of the leptotene threads is evident even from the start and as in the above two genera, the threads begin to be formed at the pole, the rest of the nucleus displaying unthreaded granules. The pachytene stage is clear and conspicuous with its thicker threads (Figs. 10 and 26) and forms, as in *Ichthyophis*, by far the most stable stage of meiosis and is one of longest duration. Gradually the polar orientation of the
threads of the pachytene nucleus is lost and soon the threads lie anyhow inside the nuclear cavity spanning it (Figs. 11 and 27). Meanwhile splits and spaces are appearing inside each thick bivalent chromosome and the duality of each of these is clear and evident (Figs. 12 and 28).

Associated with the diplotene stage has been described in the Apoda a conspicuous stage of diffusion of chromatin where the individuality of the earlier diplotene chromosomes is temporarily lost in an indistinguishable network characteristic of all the Apoda. The 'diffuse' stage has been described in a variety of plants and animals and while it appears as a more normal phenomenon in the development of the oocyte in animals, its occurrence in spermatogenesis is relatively rare but more interesting. Chickering (1928) has given a detailed account of this stage in the spermatogenesis of Belostomatidæ (Hemiptera) and his observations coincide with mine in the Apoda (1937, 1939). Chickering traces the development of diffusion in Lethocerus step by step in which the first step is marked by a separation of the two univalents at intervals, their transverse movement and a fine branching. My Fig.15 of Ichthyophis (1937) and his 59 of Lethocerus are strikingly similar. And Chickering concludes "a coarse reticulum is formed by a continuation of this process",—a statement which is very similar to my description of the process in Ichthyophis. The extension of my observations of this stage to Uraeotyphlus and now to Siphonops and Dermophis substantiate my conclusions arrived at in case of Ichthyophis and I have reasons to believe that the 'diffuse' stage is a universal character of the Apoda, following the diplotene stage and arising in the same manner as that described by me in Ichthyophis. A nucleus in the 'diffuse' stage is shown in Fig. 13.

The 'diffuse' stage is of fairly long duration at the end of which the chromosome bivalents emerge gradually from the network characteristic of the diffuse condition. The final stages of this condensation show the bivalents still long and thin and bearing a large number of transverse filamentar processes (Figs. 14 and 29). The chiasmata can be made out clearly now and it is seen that in some of the larger bivalents the chiasmata are quite large in number. This character of the large number of chiasmata in the early stages of diakinesis was noticed in Ichthyophis and also in Uraeotyphlus where 6 to 7 chiasmata were observed. In these two animals it was noticed that the number of chiasmata were gradually reduced till the largest bivalent in either form did not have more than four chiasmata in the final condition. Unfortunately I am not, on account of the unsuitability of fixation of the material, able to describe the history of the chiasmata or trace the fate of the chromosomes into metaphase.
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The division stages succeed each other rapidly, and, intercalated between the first and second meiotic divisions there is a definite stage of rest (Figs. 15 and 30) of fairly long duration as observed by me in *Ichthyophis* and *Uræotyphlus*. The spermatids are formed after the second division (Fig. 16).

The following measurements give the diameter of the nucleus in the different stages of spermatogenesis in the two genera under examination.

<table>
<thead>
<tr>
<th>Stage</th>
<th><em>Siphonops annulatus</em> microns</th>
<th><em>Dermophis gregorii</em> microns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary spermatocytes at rest</td>
<td>9 to 10</td>
<td>5 to 6</td>
</tr>
<tr>
<td>Pachytene stage</td>
<td>11·5 to 12</td>
<td>10 to 11</td>
</tr>
<tr>
<td>‘Diffuse’ stage</td>
<td>12·5 to 13·5</td>
<td>11 to 12</td>
</tr>
<tr>
<td>Diakinesis</td>
<td>15 to 16·5</td>
<td>13 to 13·5</td>
</tr>
<tr>
<td>Secondary spermatocyte</td>
<td>10 to 10·5</td>
<td>6 to 8</td>
</tr>
<tr>
<td>Spermatid</td>
<td>6 to 7</td>
<td>4 to 5</td>
</tr>
</tbody>
</table>

**Summary**

The testis structure of the two genera described here shows that it conforms to the plan outlined for *Ichthyophis glutinosus* and *Uræotyphlus narayani* except that in *Dermophis gregorii* very few testis lobes were seen. The testis locules are smaller in *Siphonops annulatus* when compared with those of the other three genera. The locules are filled with a matrix which in *Ichthyophis* and *Uræotyphlus* were determined as containing fat. In this matrix are embedded the germ cells in groups in different stages of spermatogenesis. The primary spermatogonia are found at the mouth of the duct in the locule and are believed to have arisen, as in *Ichthyophis*, from the cells lining the duct epithelium. Their nuclei may be spherical or polymorphic, the latter condition indicating a high degree of metabolic activity. Just before division, however, the nucleus resumes its spherical or oval contour. After a number of divisions, varying between six and eight, the cells,—now primary spermatocytes,—embark on the meiotic phase after a brief period of rest. The leptotene and pachytene stages follow, after which, the nucleus is marked by a ‘diffuse’ condition in which the chromosome bivalents lose their identity temporarily and the whole nucleus presents the appearance of a resting stage. When the bivalents emerge from this network, their chiasmata are clear and in the larger bivalents they are quite large in number though they are probably reduced later as in *Ichthyophis* and *Uræotyphlus*. After a brief interkinesis the second division occurs giving rise to the spermatids.
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EXPLANATION OF FIGURES

1. Longitudinal section of a testis lobe of Dermophis gregorii. The large locules are separated by thin septa and contain cell elements in various stages of spermatogenesis. Groups of interstitial cells are also seen. × 50.

2. Transverse section of a testis lobe of Siphonops annulatus illustrating the general plan of structure. × 70.

3. A part of the longitudinal section of a testis lobe of Dermophis gregorii showing a group of primary spermatogonia at the mouth of the duct. × 266.

4. Siphonops annulatus. A primary spermatagonium with a spherical nucleus and several nucleoli. Two nucleoli extruded into the cytoplasm are also seen. × 3100.

5. Dermophis gregorii. A primary spermatogonium with a slightly polymorphic nucleus × 3100.


7. Siphonops annulatus. A primary spermatogonium preparing for division. × 2266.


11. S. annulatus. Beginning of the diplotene stage. The loss of polar orientation of the bivalents is seen. × 3100.

12. S. annulatus. Early diplotene. Splits have appeared at intervals along the bivalents. × 3100.
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14. *S. annulatus*. Later stage. The bivalents have become recondensed and the chiasmata are visible. × 3100.


17. *S. annulatus*. Photomicrograph of a transverse section of a testis lobe. × 35.

18. *S. annulatus*. Photomicrograph of a testis locule showing the opening of the duct into the locule. × 200.

19. Photomicrograph of the periphery of a locule of the testis of *S. annulatus* showing three primary spermatagonia close to the septum. Their investing follicle cells are also seen. The nucleus of one of the spermatagonia (the middle cell) is preparing for division. The contrast between this cell and those on either side is sharp and clear (see text). × 700.

20. A primary spermatogonium of *S. annulatus* showing its polymorphic nucleus. × 1300.

21. *S. annulatus*. A primary spermatogonium with a polymorphic nucleus and many nucleoli. The centrosome with the centrioles is also clear. × 1400.

22. A primary spermatogonium of *S. annulatus* preparing to divide. The nucleus is vesicular with the chromatin aggregated into blocks. × 1400.

23. A binucleate primary spermatogonium of *S. annulatus*. × 1400.

24. Early spermatocytes of *S. annulatus* lying in two rows beneath the septum of the locule. × 1400.

25. *S. annulatus*. Primary spermatocytes at rest. × 1400.

26. A group of pachytene nuclei of *S. annulatus*. × 400.

27. *S. annulatus*. Beginning of the diplotene stage. The polar orientation of the bivalents is lost. × 1400.

28. *S. annulatus*. Later stage. Splits in the bivalents are clearly seen. × 1400.

29. *S. annulatus*. Nuclei showing the emergence of the bivalents from out of the diffuse stage. × 1400.