SPERMATELEOSIS IN *URAEOTYPHLUS NARAYANI* SESHACHAR AND *GEGENOPHIS CARNOSUS* BEDDOME (APODA).

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INTRODUCTION.

In 1943, the author described in detail, as the third part of his study on the spermatogenesis of *Ichthyophis glutinosus*, the spermateleosis in that species of Apoda. It was the first study of sperm formation in an Apodan example as a result of which a number of interesting facts were noticed. The form and formation of the acrosome, the changes in the nucleus, the formation of the 'middle piece', the fate of the centrioles, Golgi bodies and the mitochondria were all discussed. In the present communication, an extension of this study is made to two other species of Apoda so as to arrive at a generalised conclusion regarding the course of spermateleosis in the whole group. The two species are *Uraeotyphlus narayani* and *Gegenophis carnosus*, two Indian examples of Apoda, studies on the anatomy and cytology of which are being made in the zoological laboratories of this University. The early studies on the spermatogenesis and the chromosomes of these two species have already been made by the author (1939; 1944).

I have great pleasure in thanking Prof. A. Subba Rau for his many useful suggestions and criticisms during the course of this study.

OBSERVATIONS.

The Ripe Spermatozoon.—The ripe spermatozoa of these two species resemble that of *Ichthyophis glutinosus* already described by me and differ only in dimensions. The measurements of the different regions of the sperms are given below:—

	Uraeotyphlus narayani.	Gegenophis carnosus.	
Total length of sperm	120·0	100·0	microns.
Length of the acrosome	5·5	7·5	,,
Length of the nucleus	11·1	20·0	,,
Diameter of the nucleus	1·4	1·2	,,
Length of the 'middle piece'	5·0	7·0	,,

The sperm is surmounted by an acrosome which has the same shape as in *Ichthyophis*—that of an electric bulb, with a narrow base and an enlarged and rounded end. In all the Apoda, this form of the acrosome remains constant.

The length of the nucleus in the ripe sperm of the two species is very striking. The length in *Gegenophis* sperm is greater than that of *Uraeotyphlus*, but the diameter in the former is much smaller. I discussed the volume relations of the nucleus in five species of Apoda in an earlier paper (1943) and the figures for the two species under examination in comparison with those for *Ichthyophis* are given below:—

	Uraeotyphlus narayani.	Gegenophis carnosus.	Ichthyophis glutinosus.	
Length of the nucleus ..	11·1	20·0	8·0	microns.
Diameter of the nucleus ..	1·4	1·2	2·0	,,
Volume of the nucleus ..	20·0	22·6	25·1	c. microns.

A relationship between the volume of the spherical spermatid nucleus and that of the cylindrical nucleus of the fully formed spermatozoon is established and the whole problem of the volume relationships of the nucleus in different stages of spermateleosis in five species of Apoda is dealt with in my earlier paper on the subject (1943).

The nucleus is followed by a 'middle piece'. It is about 5·0 microns in length in *Uraeotyphlus* and 7·0 microns in *Gegenophis*. In Bouin preparations it is a clear transparent tube within which lies the base of the tail filament; but in osmic preparations, especially in Mann-Kopsch and Kolatchew preparations, a spiral mitochondrial coil is seen in close apposition to the inner wall of the tube.

The centrioles are indistinguishable as such in the ripe sperm of either species but their developmental history shows that they have become so closely incorporated with the nucleus (as in *Ichthyophis*) that their detection becomes a matter of difficulty unless efforts are made to dissociate them. The proximal centriole is in the form of a disc-like ring closely applied to the posterior end of the nucleus, and the distal centriole, as an elongated granule, has entered the nucleus through the orifice of the ring of the proximal centriole and has become embedded in it.

The flagellum bears an undulating membrane as in *Ichthyophis*, which extends over a considerable part of its length.

Spermateleosis.

(*a*) *The Centrioles.*—In the early spermatid of both the species under study the centrioles are small granules lying inside the sphere area but soon they escape from its confincs and move towards the periphery of the cell. Here they arrange themselves at right angles to the wall, one, on or near it, and the other, more internally in the cell, so that they could now be referred to as the distal and proximal centrioles repectively. From the distal centriole, a naked filament is formed, which now is almost entirely extracellular.

Then occurs the movement of the centrioles inwards, the distal one carrying its filament with it. This movement is highly co-ordinated, since it keeps the two centrioles together, and stops only when they have reached the neighbourhood of the nucleus. Once there, the proximal centriole comes in contact with it, enlarges, and is stuck as a flat plate to the nuclear membrane (Pl. X, Fig. 1).

In this movement, the centrioles may first come in contact with the nuclear membrane at any point, and may even obtain an attachment with the acrosomic vesicle itself, but slowly they migrate, again in a concerted manner, till they reach the future posterior pole of the nucleus. Only after this final posterior position is reached, the further changes in their form take place. The proximal centriole is enlarged, flattened and becomes converted into a ring (Pl. X, Figs. 2, 10, 11). The distal centriole which now becomes slightly elongated, moves through the ring and fuses with the nucleus. This is the final position of the centrioles in the two Apoda, the proximal stuck to the posterior end of the nucleus as a flat perforated disc and the distal, as an elongated granule, having passed through the orifice of the proximal centriole, has become embedded in the nucleus.

(*b*) *The Acrosome.*—The acrosome is a formation of the Golgi Apparatus. This is clear from Kolatchew preparations where the clear acrosomic vesicle is surrounded and enveloped by the Golgi elements which have secreted it (Pl. X, Fig. 8). Since the Golgi bodies occupy a juxtanuclear position almost from the start, the acrosomic vesicle occupies one of the poles of the nucleus and in close apposition with it.

The acrosomic vesicle when first formed, is spherical, and a shallow cup-shaped depression appears on the nuclear wall to receive this spherical acrosomic vesicle. The rim of the cup is strengthened by a special fibre which stains deeply. From this circular rim a number of strands, also deeply staining, meet at the bottom of the cup in a central granule.

From the central granule of the cup, there arises a deeply staining streak which projects into the nucleus. A cavity is later developed in it which becomes a deep pit.

It was already observed that surmounting the nucleus of the fully formed sperm in these two species is a bulbous acrosome. The acrosome gives off a lance-shaped pointed plug which enters this pit in the nucleus and the acrosome and the nucleus are held together in this manner.

The relationship between the acrosome and the nucleus is always with reference to such an 'acrosome seat', where the lance-shaped plug given off at the base of the acrosome fits snugly into a deep pit in the anterior end of the nucleus. A similar arrangement for holding the acrosome and the nucleus was seen in *Ichthyophis* as well.

The acrosome vesicle, when first formed, is a transparent spherical body but it soon becomes elongated and develops a central strengthening fibre. A narrowing of its base confers the bulbous appearance characteristic of it and it soon takes the definitive form shown in Pl. X, Figs. 7 and 15.

(*c*) *The Nucleus.*—At first the spermatid nucleus is spherical but it soon becomes elongated, with the anterior end slightly narrower than the posterior. It is at this stage shown in Pl. X, Fig. 9 that the centrioles have found their definitive position. The nucleus continues to elongate till the maximum is reached, which is about 17·2 microns in *Uraeotyphlus narayani* and 26·0 microns in *Gegenophis carnosus.*

Soon after this a contraction in the size of the nucleus starts, the process being the same as in *Ichthyophis.* The first stages of contraction affect not only its diameter but also its length. Before contraction, the nuclear diameter at its posterior end is much greater than that of the ring centriole attached to it, but soon the diameter of the nucleus is reduced till it is the same as that of the centriole. The contraction is one of the important features of spermateleosis and results in a considerable reduction in the total volume of the nucleus as seen in the fully formed sperm, where the nucleus has an average length of 11·1 and a diameter of 1·4 microns in *Uraeotyphlus*, and 20·0 and 1·2 microns respectively in *Gegenophis* (Pl. X, Figs. 2, 3, 4).

As the nucleus contracts posteriorly, there appears a space around it, bounded by a definite membrane. As in *Ichthyophis*, it would appear this space indicates the original extent of the nucleus before contraction and has probably been derived as a result of it. It is likely also that as in *Ichthyophis* the contracting nucleus leaves behind it a thin pellicle or membrane which occupies its original position and indicates the original extent of the nucleus. Since the contraction is not only in length but also in diameter the space is found not only behind the nucleus but also on its sides posteriorly. Correlated with this is the increase in nuclear basophily which also starts from the posterior end and moves forwards till it pervades the whole nucleus (Pl. X, Figs. 2, 3, 4, 5 and 6).

Later stages of spermateleosis show that this space, bounded, as we have seen, by a membrane, moves backwards and occupies a totally post-nuclear position and is fitted to the nucleus in the form of a tube (Pl. X, Figs. 5, 6, 14). Through this tube the axial filament passes. The fate, therefore, of the post-nuclear space is not far to seek. It becomes the tube of the 'middle piece' of the sperm and its wall becomes the wall of the tube. At first the tube is empty, but later the mitochondria migrate into it from the cytoplasm and organise themselves to constitute the special investment around the axial filament.

(*d*) *Golgi Bodies.*—That the acrosome arises in relation with the Golgi bodies has been known for a long time and in the Apodan spermatid, soon after the secretion of the acrosomic vesicle, the Golgi bodies migrate backwards and take up a posterior position. So far as I have been able to ascertain, they do not take any further part in shaping the sperm and are cast off with the residual cytoplasm (Pl. X, Figs. 4, 5, 6, 13, 14).

(*e*) *Mitochondria.*—The mitochondria in the Apodan examples studied are at all stages granular and in the early spermatid scattered in the cytoplasm. This arrangement continues till a late stage in development and as the nucleus elongates, the mitochondria invest it on all sides. It is when the tubular 'middle piece' sheath is being formed that they spring into activity and by the time the sheath is completed, all the mitochondria have collected posteriorly. They now migrate into the tube of the 'middle piece' and arrange themselves on its inner wall to form the spiral investment so characteristic of the fully formed sperm (Pl. X, Figs. 4, 5, 6, 8, 12, 14).

Discussion.

The foregoing account of the sperm formation in *Uraeotyphlus narayani* and *Gegenophis carnosus* is, in the main, identical with that described for *Ichthyophis glutinosus.* In this respect the Apoda exhibit a uniformity and homogeneity rarely seen in any other group of animals.

The sperm formation in Apoda, as is clear from the examination of these three species, is very different from that occurring in the other two groups of Amphibia. The adult sperm itself resembles the Anuran sperm rather than the Urodelan, a curious feature, for in a large number of anatomical and cytological characters, there is a close similarity between the Apoda and the Urodela. But this diversity between the Urodele and Apodan sperms is probably due largely to the highly atypical character of the sperms of Urodeles, which are quite unlike those of any other animal. The occurrence of both centrioles in close proximity with the nucleus as well as the post-nuclear mitochondrial aggregation in the 'middle piece' are anuran features, while there is nothing in the Apoda (for that matter, in any other animal) like the great modification of the centrioles found in the Urodele sperm.

But this similarity is not too close. In the Anura, the centrioles are found merely as granules behind the nucleus; they undergo a modification in the Apoda. The flattening of

the proximal centriole to form a disc and the remarkable manner in which it is fitted to the posterior end of the nucleus are features unique in the Apoda and are found in all the examples examined so far. In the centre of this disc is an aperture and through this aperture, the distal centriole (which also slightly elongates) moves forwards into the nucleus and becomes embedded in it. The intimate relation into which the two centrioles get with the nucleus of the adult sperm is so great that in an ordinary preparation of the spermatozoon not specially made to demonstrate the centrioles, it is impossible to see them. But in well-made preparations, a faint space can be made out between the disc-shaped centriole and the nucleus. The anuran condition, on the other hand, is far simpler. The two centriolar granules lie one behind the other (Broman, 1900, 1901, 1917; King, 1907; Champy, 1913, 1923). The condition found in the Urodela, with its great modification of the centrioles is unparalleled, so far as we know, in the animal kingdom.

In the matter of the acrosome, no complete observations are available with reference to the Urodela and Anura. Gatenby (1931) has described the formation of an acrosome seat in *Desmognathus* spermateleosis but has not carried his observations far. The whole process of acrosome formation and the manner of the attachment of the acrosome with the nucleus of the sperm have been studied by me in *Ichthyophis* (1943) and those observations have now been extended to the two other species of Apoda under examination. There is a remarkable uniformity in this group in the matter of shape, form and development of the acrosome and many of my findings in *Ichthyophis* have received confirmation in *Uraeotyphlus* and *Gegenophis*. In the Urodela and Anura on the other hand, the form, shape and size of the acrosome are subject to great variation.

In all the Apoda examined by me, the acrosome of the sperm is in the form of a bulb-shaped body planted on the anterior end of the cylindrical nucleus. While this uniform shape of the acrosome is itself of sufficient interest, the manner of its attachment to the nucleus is even more interesting. No work has been done on this aspect of sperm formation apart from the observations of Gatenby (1931) on the early history of the acrosome formation in *Desmognathus*, and literature does not reveal any intensive studies on this aspect of the question. That the Golgi bodies secrete the acrosome has been accepted on all hands, but how it is related to the nucleus after its formation is a matter which has received very little attention.

That the acrosome is fitted to the nucleus with reference to the acrosome seat at its anterior end has already been established by me in *Ichthyophis* and my findings in the two species under study confirm those observations. The details of the formation of the acrosome seat and the attachment of the acrosome to the nucleus have been described already and throughout the Apoda this type of acrosome seat is seen as a uniform structure.

There is no doubt that this type of acrosome seat is correlated with the form of the acrosome itself and since the latter has a uniform shape in Apoda, the former may also be expected to show a uniformity in its form and formation. But in other animals, where the acrosome is subject to great variation in its form, a study of the acrosome seat should yield interesting results.

The nucleus also offers points of interest and uniformity in the Apodan series. The first change in the nucleus relates to its form and size. The spherical nucleus of the spermatid becomes the elongated cylinder which is the nucleus of the ripe sperm and correlated with this is also a great diminution in its volume. In the two species under examination the reduction in volume of the nucleus during spermateleosis is as much as 90%. It is also of some interest to note that the other Indian species, *Ichthyophis glutinosus*, falls in a line in that it also undergoes a 90% reduction in the volume of its nucleus during spermateleosis (Seshachar, 1943).

Associated with this diminution in size, indeed, because of it, a space appears posteriorly and all my observations go to show that this space becomes the 'middle piece' of the sperm. The space which at first represents a small clear area behind the nucleus gradually enlarges and is organised to form the tube into which the mitochondria migrate and arrange as a spiral thickening over the axial filament. Similar observations were made in the spermateleosis of *Ichthyophis* where the whole problem has been discussed by me (1943*a*).

Of the other cytoplasmic inclusions, the Golgi bodies, after secreting the acrosome, break up and move backwards to be cast off in the sloughing residual cytoplasm. It is practically certain that in the fully formed sperm no Golgi bodies are seen. Evidently their only function is to form the acrosome. The mitochondria, on the other hand, remain as permanent structures of the sperm and occupy the 'middle piece' region as a spiral thickening around the base of

the axial filament. The 'middle piece' itself, as in *Ichthyophis*, is a cylindrical tube fitted to the posterior end of the nucleus.

SUMMARY.

A study of the spermateleosis in *Uraeotyphlus narayani* and *Gegenophis carnosus* shows that the process agrees closely with that in *Ichthyophis glutinosus* already described by the author (1943*a*) revealing the following important points occurring uniformly throughout the group.

A. The acrosome of the ripe sperm is bulb-shaped and its stalk is fitted on to the nucleus in relation with an acrosome seat. This is in the form of a deep pit in the centre of the nucleus into which the acrosome sends a lance-shaped plug which fits closely and which holds the acrosome and nucleus together.

B. Of the two centrioles of the spermatid, one becomes a flattened ring and is pressed against the nucleus posteriorly. This is the proximal centriole. The distal centriole becomes slightly spindle-shaped and moves forward through the ring into the nucleus and fuses intimately with it. The axial filament is given off from the distal centriole.

C. The 'middle piece' in the Apoda is a cylindrical tube inside which the mitochondria are arranged spirally.

D. The nucleus is greatly consolidated and, in the fully formed sperm, is cylindrical.

E. The Golgi bodies, after they have formed the acrosome, move backwards and are sloughed off.

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EXPLANATION OF PLATE X.

FIG. 1.—A developing spermatid of *Uraeotyphlus narayani* showing the acrosomic vesicle planted in the acrosome seat on the nucleus. The proximal centriole is flattened and attached to the nuclear wall; the distal is granular and has given off the axial filament. × 2250.

FIG. 2.—Later stage of the same showing the formation of the post-nuclear space. The ring-shaped proximal centriole is seen posteriorly through which the distal centriole is passing forwards into the nucleus to become embedded in it. × 2250.

FIG. 3.—The post-nuclear space is better defined and the nucleus is contracting rapidly. × 2250.

FIGS. 4, 5, and 6.—Kolatchew preparations of the developing spermatids of *Uraeotyphlus narayani* to show the development of the post-nuclear space into the 'middle piece' tube of the sperm. The Golgi bodies and the mitochondria are aggregated behind the nucleus. Only the latter migrate into the 'middle piece' tube. × 2250.

FIG. 7.—A ripe sperm of *Uraeotyphlus narayani* showing the different regions. × 1125.

FIG. 8.—A Kolatchew preparation of the early spermatid of *Gegenophis carnosus* showing the development of the acrosomic vesicle in connection with the Golgi bodies. The mitochondria are scattered in the cytoplasm. × 2250.

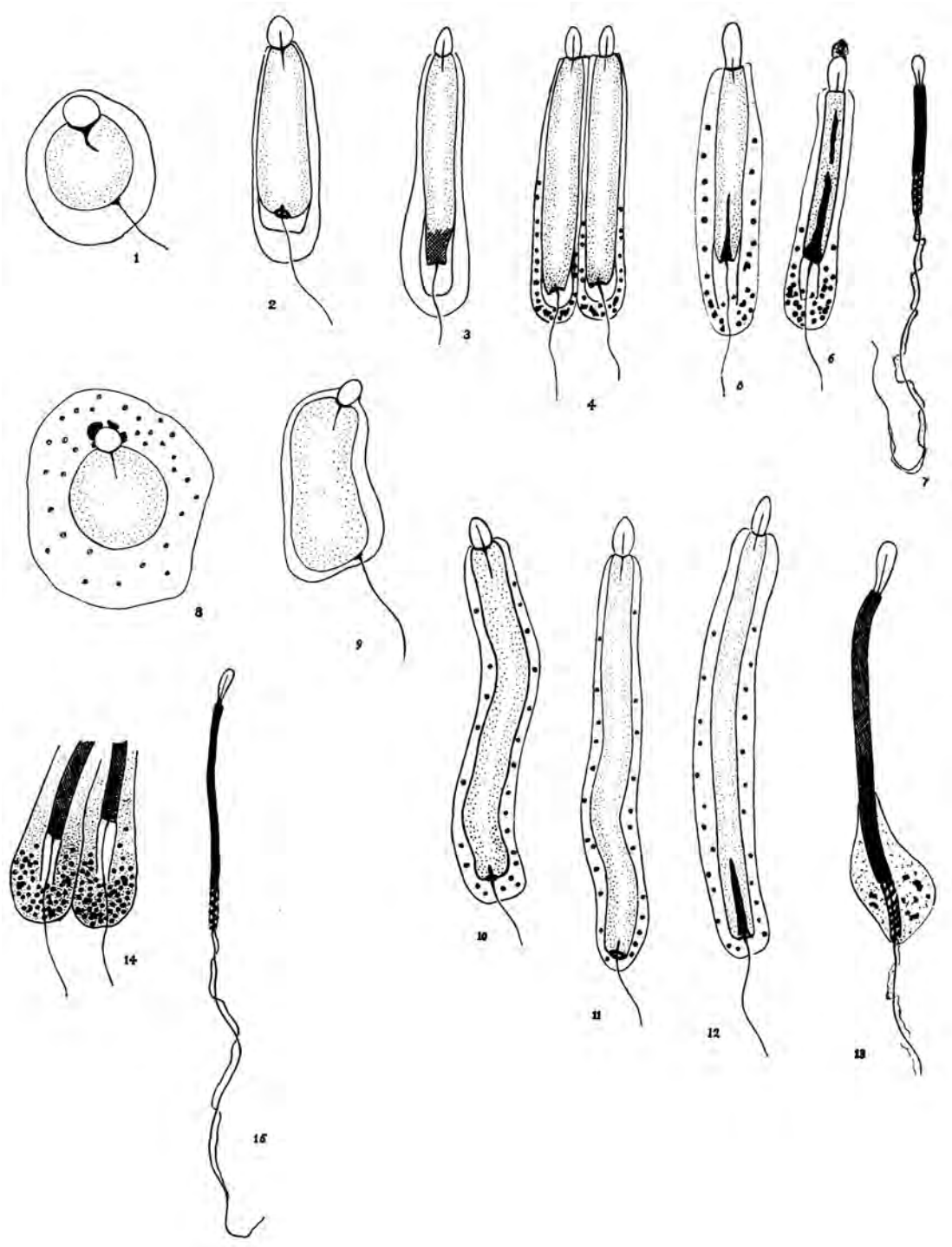
FIG. 9.—Later stage of the same showing the acrosomic vesicle, acrosome seat and the two centrioles. The nucleus has elongated. × 2250.

FIGS. 10, 11, 12.—Stages in the spermateleosis of *Gegenophis carnosus* showing the gradual elongation of the nucleus. The two centrioles are clear, the proximal as a ring stuck to the posterior end of the nucleus, the distal as a spindle-shaped body carrying the filament and embedded in the nucleus. × 2250.

FIG. 13.—A nearly ripe sperm of *Gegenophis carnosus* showing the sloughing off residual cytoplasm in which the Golgi bodies are present. The mitochondria are found inside the 'middle piece' as a spiral thickening. × 2250.

FIG. 14.—Posterior view of two developing spermatids of *Gegenophis carnosus* showing the formation of the 'middle piece' tube. The Golgi bodies and mitochondria are aggregated posteriorly. × 2250.

FIG. 15.—A ripe sperm of *Gegenophis carnosus* showing the different regions. × 1125.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