Spermiogenesis in *Lumbricus herculeus*
An Electron Microscope Study*

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PLATES 22 TO 27

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**ABSTRACT**

Small pieces of the sperm sacs of *Lumbricus herculeus* were fixed for 4 hours in chrome-osmium, embedded in methacrylate, sectioned with a Porter-Blum microtome, and studied with a R.C.A. EMU-2C electron microscope.

Each spermatid of a group developing synchronously is attached by a cytoplasmic strand to a common nutrient protoplasmic mass. This mass contains mitochondria and yolk bodies but is anucleate.

The proximal centriole, that is, the centriole nearer the nucleus, is at first associated with a small peg which becomes firmly attached to the nuclear membrane. Later these two bodies become separated during the development of the middle-piece which is differentiated in the usual manner from a nebenkern formed by the fusion of 6 or 7 mitochondria.

The acrosome develops in relation to the dictyosome (Golgi body), itself composed of 8 or more individual flattened sacs and situated in the cytoplasm opposite the point of attachment of the spermatid to the nutrient mass. Soon after its formation, the acrosome becomes incorporated into a cytoplasmic appendage or acrosome carrier. The carrier moves from its original position, along the lateral border of the elongating nucleus, to the distal margin of the nucleus where the acrosome is deposited. No evidence was found of a centriole located at the point of junction between nucleus and acrosome as suggested by earlier workers.

**INTRODUCTION**

Two able French microscopists, E. Chatton and Odette Tuzet (1941, 1942, 1943) have investigated oligochaete spermiogenesis, which they state has often attracted the “travaux pratiques des débutants,” and their work, and our electron micrographs, provide for interesting comparisons. We cannot claim to have cleared up all problems in this spermiogenesis, especially since we have encountered some puzzling features in the metamorphosis of the spermatid into ripe spermatozoon. A relatively large number of cells were examined without obtaining all the necessary stages, the very thin sections used by the electron microscopist naturally increasing the difficulties of the spermatologist.

**Materials and Methods**

The earthworms, *Lumbricus herculeus*, were obtained in Dublin, Ireland, in early September, and fixed in chrome osmium solution at pH 7.2 (Dalton, 1955). The pieces were stored in 70 per cent ethanol, embedded in methacrylate, and cut with a Porter-Blum microtome; the electron micrographs were taken with a R.C.A. EMU 2-C electron microscope.

**PREVIOUS WORK**

E. Chatton and O. Tuzet (1941) have given the most recent light microscopical account of the spermiogenesis of *Lumbricus*. According to them, there are fifteen to twenty mitochondria in the young spermatid, which come together to form four to six mitochondrial spheres (see Text-figs. J. BIOPHYS. AND BIOCHEM. CYTOL., 1959, Vol. 6, No. 1
One of these groups passes out into an outgrowing protoplasmic bead, which becomes drawn out and finally detached (Text-figs. 3 and 4, \textit{mx}). This is the \textit{Auszerkorper} or mitochondrial reject of Hesse (1909) which takes no part in the formation of the middle-piece. Those mitochondria that remain in the cell, adhere to the posterior end of the nucleus, and form the middle-piece in conjunction with the posterior centrioles. A short time before the acrosome is formed, a curious cylindrical acidophile capsule, \textit{cs}, in Text-figs. 5 to 8, appears in contact with the anterior centriole. This "corpuscle spheroide" slips down (Text-fig. 7) and ultimately disappears as the sperm ripens. This spheroidal corpuscle appears only in 50 per cent of spermatids. According to Chatton and Tuzet, the true acrosome (\textit{a}, in Text-fig. 5) appears in front of the spheroidal corpuscle, grows, and forms a flagellum in front (Text-figs. 6 to 8), which is later absorbed.

Chatton and Tuzet thus claim three deviations from the usual account of spermatogenesis: (a) The appearance of a protoplasmic corpuscle (Text-figs. 3 and 4) by which part of the mitochondria are eliminated; (b) The transitory existence of a peculiar spheroidal corpuscle at the anterior end of the spermatid, its migration down the spermatid, and its absorption as it approaches the middle-piece and lastly, so far as we can understand them, (c) the presence of a centriole somewhere near the acrosome, and at the anterior end of the spermatozoon (presumably \textit{c?} in Text-fig. 9). The following year (1942), these authors described in two lumbricids, \textit{Allolobophora chloritica} and \textit{Lumbricus herculeus}, a peculiar "veritable maladie nucleaire du sperme," associated with the nucleolus, so that half the sperms are anucleolate or normal, and the others, subnucleolate and perhaps non-functional. These authors describe the nucleolus as taking up a central position, passing forward, and in 50 per cent of cases as peeling out and being eliminated. The site from which the nucleolus peeled out is left as a space. Later Tuzet alone (1946) further studied atypical spermiogenesis in lumbricids.

In 1946, A. T. Brice, Roza P. Jones, and J. D. Smyth first used the phase contrast microscope to show the dictyosome in the living spermatids of \textit{Lumbricus sp}. They stated that the Golgi apparatus appears as a dense black crescent.

RESULTS

Text-fig. 10 is a semi-diagrammatic figure illustrating one of the interesting features of \textit{Lumbricus spermiogenesis}, that of the central nutrient mass (CNM) to which all spermatids of one clone connect. In this case the cells attached to the mass are nearly ripe spermatids. (A micrograph of one younger spermatid so attached is shown in Fig. 12.) The junction with the nutrient mass is marked by an external fur-like covering, \textit{FU}. Mitochondria, \textit{M}, and large granules or vacuoles, \textit{Y}, occur within the nutrient mass.

\textbf{Early Spermatids:}

The earliest spermatids are in Figs. 3 and 4, both from the same clone. The centriole, \textit{C}, is a hollow, short, tubular structure, usually surrounded by adherent or adjacent small granules.

No mitochondria were cut in Fig. 3, but these are shown in Fig. 4, from a neighboring cell. The Golgi apparatus is at \textit{G}, and consists usually of from 8 to 12 flattened sacs. There appears to be no special aggregation of vacuoles within or
Text-Fig. 10. Part of the central common nutrient mass (CNM) showing a complex network of attachments of nearly ripe spermatozoa to the central mass. This contains various yolk spheres and vacuoles (Y), and mitochondria (M), but no nucleus. The acrosomes (A) and upper ends of the sperm nuclei (N) are cut across transversely or obliquely. The junctions between cells and central mass have a collar of electron opaque material (FU). Five spent acrosome carriers are seen at (K). Refer to Text-fig. 14 for a cell at approximately this stage with spent acrosome carrier, cell attachment, etc.

Abbreviations for Text-Figs. 10 to 15.—A, acrosome; C, centriole (C1 proximal, C2 distal); D, dictyosome, acroblast, or Golgi apparatus; F1, flagellum; FU, fur-like collar around junction of cell and central common nutrient mass; G, carrier vesicles; H, unidentified body; K, acrosome carrier; M, mitochondria and mitochondrial nebenkern or middle-piece; N, nucleus; NM or CNM, central common nutrient mass; P, postnuclear (centriole adjunct) peg; T, acrosome carrier tube; V, acrosome carrier vacuole; X, anlage of adnuclear strip (Fig. 11) and strip in Fig. 13; Y, yolk or nutrient bodies; Z, acrosome tip.

between the lamellae, but there are scattered vesicles associated with the Golgi apparatus. These probably are of the same nature as the lamellae, since they show a constant and intimate relationship to them.

The mitochondria are unusual. In Fig. 7, there are seven profiles and in Fig. 16 at a later stage, there appear to be six forming the fused mitochondrial nebenkern (cf. Chatton and Tuzet). This is probably the true number as no free mitochondrial profiles are identifiable when the mitochondrial nebenkern is formed. Furthermore, only single mitochondrial profiles are visible on either side of the mid-line in longitudinal sections of the nebenkern (Figs. 12 and 17). In Figs. 1, 8, and 15 it is evident that the mitochondrion consists of a moderately electron opaque matrix containing two or three less electron opaque compartments each bordered by a dense membrane. The mitochondrion at this stage are ellipsoidal. In stages earlier than the spermatid, we have found filamentous mitochondria, and in these the cristae run along the length of the mitochondria. In favorable cases, the characteristic outer membrane can be identified.

Fig. 1 may be taken as representative of the next stage in spermiogenesis. The cell is definitely oriented, the centriole (C) has divided into parts, one of which has approached the nucleus, but is separated therefrom by a discoidal body marked P. Three of the mitochondria have approached the proximal centriole, two others still being
further away. At the edge of the cell at K is a body destined to become associated with acrosome formation.

The Postnuclear Peg:

In Fig. 21 a small body (P) can be seen. Examination of Figs. 1, 7, 16, and 17 shows that just behind the nucleus, and in between the hollow mitochondrial nebenkern, there is always such a body. It may be considered that this is a centriole—the proximal centriole—but this peg is too small to be a centriole, is not hollow, and is not connected to the flagellum. The true proximal centriole is shown in Fig. 11 (C), and this can be compared with the centriole in Fig. 3 (C). The anlage of this peg-like body is seen in Fig. 7, where the proximal centriole (C) also may be seen. We have, therefore, assumed that the proximal centriole accompanies the peg to the nucleus (Fig. 1) and when the peg is in situ, the mitochondria collect around, and the proximal centriole withdraws to the position seen in Fig. 11, which it never leaves (Figs. 13, 14 (C)). These figures also show the distal centriole (C2).

The Acrosome and Associated Vesicles:

In Lumbricus, the acrosome, as in all other known flagellate spermatozoa, develops in connection with the dictyosome, acroblast, or Golgi body (G). In Fig. 18, the acrosome (A) appears to be fully formed, lying in a nest made by folded over lamellae. At this stage the acrosome is always ovoid or ellipsoidal, and it contains some of the small vacuoles which previously lay free in the locus of its formation. In this figure, the outer region of the acrosome is more electron opaque, the mitochondrial nebenkern is well formed and adherent (M), and closely adherent to the nucleus.

On the analogy of what is known to occur in other animals, it may be assumed that the acrosome is simply carried up and fixed on the anterior end of the nucleus, the acroblast subsequently drifting down as the spent Golgi remnant. This simple and natural assumption is, however, incorrect. In Fig. 16, the acrosome has drifted away from the acroblast (G) and appears to be entering a tube (K) which lies within a clavate projection from the cell body. In this figure, the mitochondrial nebenkern is formed and is adherent to the nucleus, and the flagellum must lie towards the observer. In Fig. 9, a similar arrangement holds in the cell to the right, the body (K) not being implicated in the mitochondrial nebenkern (M) or centriole (C).

Figs. 5 and 6 A to C show four cells providing earlier stages in the appearance of this peculiar body. In Fig. 6 A, the body (K) near the edge of the cell becomes opened to form the flattened bag seen in Figs. 5 and 6, B and C. Going back to an earlier stage, we find this body as an almost solid object at K, in Fig. 1. In the original micrograph two small arms can be seen projecting forwards, and presumably forming the anlage of the parts seen in Fig. 6 C (K). In Text-figs. 11 to 14 an interpretation of these early stages has been given. It might be suggested that the object K in Figs. 5 and 6 is merely the acrosome, but we have several stages similar to that in Fig. 16, where the acrosome and the body K are manifestly separate structures. So far as we can understand, after the acrosome has passed into the body K (from this point on called the acrosome carrier) the whole object including a vacuole, a granular mass, and a reception tube plus the acrosome, grows as shown in Fig. 2 (K) where each spermatid has a globular carrier lying in the posterior region of the cell. It will be noted that in this figure, the mitochondrial nebenkern is well formed and adherent to the nucleus, and the flagellum has passed out of the cell. In Fig. 16 there is a slightly earlier stage cut at right angles to that in Fig. 2. In Fig. 9 right, the carrier (K) is cut across, but in the cell to the left it was missed. However, the emergent tail does show at C2.

It will now appear that Chatton and Tuzet (cf. Text-figs. 3 and 4) were correct in describing a protoplasmic projection at these stages of spermiogenesis.

Now in Figs. 2, 5, and 6, 9 to 11, and 16 and 17, this acrosome carrier is shown at various stages. By comparing Fig. 2 (upper), with Fig. 16 the carrier is seen to consist of at least three parts—a vacuole, a granular mass, and a reception tube, plus the original acrosome, all enclosed in a single wall, which appears to be the cell membrane.

In Fig. 20, the carriers now lying at the anterior end of the spermatids contain a single vacuole, and a slight electron opaque coagulum—in other words, something has gone from them. In Fig. 19, the ripening sperm head has an acrosome, with dark fore and aft parts, corresponding to that in Fig. 16 (A). The spermatozoa in Fig. 20 are nearly ripe, and the acrosome has lengthened considerably. No Golgi remnant or spent dictyosome has ever been seen at the anterior end of the bundle of ripening spermatids, and we conclude that the acrosome carrier, once in a posterior position, has
TEXT-FIgs. 11 to 15. Semi-diagrammatic interpretation of spermiogenesis of *Lumbricus*. For simplicity, the timing of development of the various parts is made somewhat out of step especially in Fig. 13, where the acrosome carrier (K) does not ascend till later. In Fig. 11 the acrosome (A) has left the Golgi apparatus or acroblast (D) and is passing into the reception tube (T) with some vesicles (G). In the postnuclear region the centriole adjunct peg (P) is in situ in front of centriole 1. The (six) mitochondrial spheroids are in position, and centriole 2 has passed back to the edge of the cell and has sent out the flagellum (F1). Adherent to the nucleus is an aggregation (X) which later spreads along the nucleus as a strip on one side only as in Fig. 13. Each cell is attached by a narrow neck to the central common nutrient mass (NM). There is an unidentified body at H. In Fig. 13, the acrosome carrier is passing up to the anterior end of the cell, leaving the Golgi apparatus behind. As the cell narrows and lengthens the saccules or lamellae of the Golgi apparatus tend to become more or less separated. The mitochondrial nebenkern is closing in Fig. 12, a string of granules connecting the peg (P) to centriole 1. By the stage in Fig. 13, a new part of the flagellum (F2) is established between the two centrioles, but the middle-piece now lies in front of centriole 1. In Fig. 14, the acrosome carrier has given up its acrosome, which is now fixed to the nucleus, an empty space being left in the carrier. In Fig. 15, there is the ripe sperm, with acrosome tip, acrosome, nucleus, middle-piece and flagellum; length of parts approximate.

rapidly migrated up the cell, and has given up part of its contents. We use the word rapidly, because we have found few intermediate stages of migration. In Fig. 10 there is a spermatid intermediate in development between those in Figs. 16 and 20; the nuclei of the sister cells in this case stretch across the width of two plates, only a very small part being shown in Fig. 10. Here, however, the granular material and one vacuole are shown (Compare Figs. 2 and 10). The nature
of the dark object between the cytoplasm of this spermatid in Fig. 10 and the globule is not known for certain, but is possibly a sperm tail and/or a centriole. Reference to this is made below.

In Fig. 7, no acrosome is present in the section, but to the left of the letter G, there is a group of especially electron opaque microvesicles and two other larger ellipsoidal vacuoles. These also occur in Figs. 5 (AX) and in Fig. 16 large oval vacuoles are present. In Fig. 15, the acrosome (A) is partially formed and the acrosome wall appears to be enclosing various microvesicles seen more clearly in Fig. 18. Later in the history of the acrosome, these vesicles appear to dissolve or coalesce so that in the fixed acrosome (Figs. 19 and 20) the contents consist of a faint coagulum.

**Centrioles and Associated Bodies of the Mitochondrial Nebenkern:**

The presence of a body, the postnuclear peg, associated with the centriole (see Fig. 1, P) has already been mentioned. When the mitochondria assemble to form the nebenkern (Figs. 7 and 16), this peg is always lying inside and can be seen in all sections passing through the middle of the mid-postnuclear region. The peg is seen in Figs. 1, 2, 16, and 17 (cell at right). It has been suggested that the postnuclear peg (Fig. 7, P) is present before it attaches to the postnuclear region. There is another possible explanation—this peg may be a bud from the centriole. On a priori grounds we do not believe that the partly intranuclear peg originates from the centrioles. Such postnuclear or centriole adjunct bodies are now known from Orthoptera (Gatenby and Tahmisian, in press), and here they definitely are not budded from the centriole.

The postnuclear peg is not the only body associated with the mitochondrial nebenkern. There are also vesicles or granules marked T (Figs. 12 and 17). In Fig. 7 and Fig. 15 at an earlier stage, the bodies marked T are believed to be the anlagen of this material which later becomes incorporated in the forming mitochondrial nebenkern. It is possible that the body marked P in Fig. 16 is of a similar nature. In Fig. 17 (right), the postnuclear peg (P) is supported by similar material.

**Centriole and Acrosome Carrier:**

Large numbers of examples of the acrosome carrier such as those shown in Fig. 2 (K) have been found which do not contain a centriole, but in Fig. 10, a fairly late spermatid, the acrosome carrier, does contain a body (XF) which may represent a tangential section through a distal centriole and part of the flagellum; the latter is bent towards the posterior end of the cell. This suggestion is made, because while this body is in the position one might expect for the acrosome at this stage, it does not possess the typical form and density of the latter (A in Fig. 16).

In Fig. 8 the centriole at the edge of the cell does not contain a central filament as does the cross-section of the flagellum just above it. In Fig. 9 (left), the flagellum (or centriole), still intracellular, does contain the central filament. We believe that the distal centriole first sends out the flagellum, and the connection between the two centrioles seen in Fig. 14 is established later as the cell elongates.

**Nucleus and Nuclear Membrane:**

In Fig. 1, the nucleus has the double membrane so often seen, but later when the spermatid is at the stage of pyramidal shaped middle-piece, the curious serrated nuclear membrane appears as in Fig. 11. This appearance is caused by sections through short tubules lying parallel on the nuclear surface. Such tubules are seen on the left of Fig. 11 in situ on the nuclear membrane. "In toto" they probably correspond to the caudal sheath of vertebrate spermatids. The origin of the tubules is doubtful, but it is possible that they are of cytoplasmic origin since they seem to appear first as spheres or tubules derived from the outer (cytoplasmic) nuclear membrane. In Fig. 18, to the left of the letter M two such spheres are apparent on the nuclear membrane. Similar areas can be seen in Fig. 16 to the right of the letter N. At a later stage as in Fig. 13, the surface of the nucleus becomes smooth, an intermediate stage being seen in Fig. 10.

Turning now to the nuclear contents, the early spermatid has the usual reticulate arrangement (Fig. 1). The finely granular condition which subsequently develops (Figs. 11 and 12) is followed by a coarsely granular phase (Fig. 20), and finally a completely electron opaque smooth condition marks the end of spermiogenesis (Figs. 13, 14, and 19).

**DISCUSSION**

We do not propose here to discuss our findings in the light of recent electron microscopical studies of invertebrate spermatogenesis, but we must consider the work of Chatton and Tuzet. In their short paper, they did not mark all the various
parts depicted in our copy of their figures given in our Text-figs. 5 to 9. Thus we are not clear as to the exact location of the anterior centriole referred to by them as lying at the front end of the ripening spermatid. We believe that our Fig. 14 shows the two centrioles in a fairly normal position and these, as in Fig. 1, appear normal. Nothing that could be interpreted as a centriole has been found at the acrosome region in such stages as those in Figs. 19 and 20. If there is a centriole here, it must have altered its usual morphology from that of a hollow short tube. In Fig. 10, the body XF may be a centriole and part of the flagellum. If so, we regard it as having been caught up by the acrosome carrier which has pulled the flagellum with it. Though this is the only case of its kind we have found, we have purposely included it in our micrographs, in view of Chatton and Tuzet’s interpretation. The lack of serial or thick sections makes such points difficult to settle. In the cells in Figs. 6 A and B, the anlage of the vacuolar part of the acrosome carrier, contains a bead (B) but this is certainly not a centriole, as in various micrographs the true centrioles are seen away from this site. In Text-figs. 11 to 14 we have given our present view as to what is happening in this region. There is one point which is not clear to us—the stages subsequent to that in Fig. 1, where the original centriole has divided into two as normally happens in flagellate spermiogenesis. In Fig. 8, the flagellum is already out of the cell, but we are not sure about the connection between the two centrioles at this period. We know that not long after this period one centriole comes to lie in the position shown in Fig. 11, and we also know that in Figs. 13 and 14 two centrioles, presumably the proximal and distal, are seen. We do not believe that in Fig. 16 the acrosome carrier (K) has any regular topographical relation to the centrioles, one or both of which can usually be seen in a different locus as in Fig. 9 (right).

The most satisfactory information we have on the relation between the two centrioles is seen in Fig. 9 (left), where we believe part of the proximal centriole is cut at C, (cf. Fig. 11, C), and the distal centriole is at the lower position (Cs).

As to the functions of the parts of the acrosome carrier, it would appear that the thick walled right hand vacuole in Fig. 2 (lower) is the acrosome and the other marked K is the spent vacuole seen at a late stage in Fig. 20 (K). But the nature and use of the granular part seen in Fig. 10 and Fig. 16 is unknown to us. The granular part is not seen in the rejects in Fig. 20 (K) but it does appear in other micrographs such as those from which Text-fig. 10 was compiled. The acrosome in Fig. 20 has a tip (Z) the origin of which is not known, and in the spermatid in the middle of this micrograph the protoplasmic anterior bead has an inclusion. Neither this, nor the tip (Z) resembles a centriole.

Regarding the mitochondria, these may be fifteen or twenty in the early spermatid as claimed by Chatton and Tuzet, but we have never seen this. We have only found about six, and we have never found them aggregated into two groups as claimed by the French authors. It seems clear that the acrosome carrier (K) in our micrographs is the protoplasmic bead which is claimed by Chatton and Tuzet to peel off the rejected mitochondria. We make these observations well knowing that the light microscopist could not expect to see clearly objects which we have studied not always easily at ×70,000. It seems likely that the filiform acrosomes of Chatton and Tuzet are the shrunken and drawn out attachments of the spermatid to the central nutrient mass, and that the migrating “corpuscle sphéroïde” of these authors probably is the acrosome carrier going up and not coming down as suggested by Chatton and Tuzet. In one case, we found an oval space in the ripening nucleus of the spermatid but this did not occur in other micrographs. We have no observations on the “veritable nucleolar malady” of the French authors, and found no evidence of this.

It may be thought that such a variation as the formation of the acrosome carrier is a unique process in animal spermatogenesis, but this is not so. R. Devine has recently shown (personal communication, 1958) that in the locustid, *Melanoplus differentialis*, the acrosome becomes adherent to the nucleus in the normal way after leaving the acroblast, then while remaining attached to the nuclear membrane, approaches the cell membrane where a reaction takes place producing a special anchoring or covering device formed outside the plasma membrane. This and the behavior of the lumbricid acrosome carrier could not be seen properly with the light microscope. No doubt our knowledge of such cases will increase as more work is done at the high magnification of the electron microscope in the spermatogenesis of other animals.

Acknowledgment: We thank Professor R. A. R. Gresson of the Queens University, Belfast, for kindly sending us a list of previous literature on oligochaete spermatogenesis, which was of considerable assistance.
LUMBRICUS SPERMOGENESIS

These references include Bloomfield (1880), Sabatier (1882), Calkins (1894, 1895), Erlanger (1896 a and b), Foot and Stroebell (1902, 1903), Brasil (1905), Bugnion and Popoff (1905), Depdolla (1905, 1906), Hatai (1900), Hesse (1909), and Stephenson (1930).

LITERATURE


EXPLANATION OF PLATES

Legends for Plate Figures

A, acrosome; AX, proacrosome vesicle; B, granule within acrosome carrier anlage; C, centriole; F, fur-like surround on part of the connective between cell and central nutrient mass; G, dictyosome or Golgi body; J, junction between cell and central nutrient mass; K, acrosome carrier; M, mitochondrion or mitochondrial middle-piece; N, nucleus; P, postnuclear peg, or centriole adjunct, or postnuclear pin; T, granules around postnuclear peg and mitochondrial nebenkern; X, unidentified body; XF, possible centriole and flagellum; Y, central nutrient mass; Z, formed tip of the nearly ripe spermatozoon.

PLATE 22

Fig. 1. Spermatid at time of division of centriole (C) into proximal and distal parts. Note postnuclear or centriole adjunct peg (P). At (K) is the supposed earliest anlage of the acrosome carrier (see K in cells in Figs. 6 A, B, and C). Approximately X 32,000.

Fig. 2. Stage of first complete coalescence of mitochondria to form nebenkern. At (K) (above) the acrosome carrier, probably belonging to this cell is shown. Approximately X 32,000.

Fig. 3. At (C) the undivided early spermatid. Approximately X 32,000.

Fig. 4. Mitochondrial profiles in a cell from the same clone as that in Fig. 3. Six profiles are visible. Approximately X 32,000.
(Gatenby and Dalton: *Lumbricus* spermiogenesis)
PLATE 23

Fig. 5. The anlage of the acrosome carrier now begins to open and form a bag, as in Fig. 6 B. At AX there is one of the vesicles which later coalesce to form the mature acrosome. Approximately × 32,000.

Fig. 6. In A and B there are two daughter cells showing stages in formation of carrier (K). Compare Fig. 6 A with 6 C. The components of the carrier visible in the cell in 6 C can be seen in 6 A. A following stage is seen in Fig. 16, in this case oriented as the carrier anlage in Fig. 6 A. Approximately × 32,000.

Fig. 7. The postnuclear or centriole adjunct peg is at P. Its sharp end above becomes embedded in the nucleus. Compare this with the peg in Fig. 1 and Fig. 17. Note the centriole at C, and the profiles of seven mitochondria. Approximately × 32,000.

Fig. 8. Outgrowing root of the incipient flagellum from presumably the distal centriole. There is no central filament inside the centriole. An infolding of the cell membrane at this locus is commonly found at this stage. Approximately × 32,000.
(Gatenby and Dalton: *Lumbricus* spermiogenesis)
PLATE 24

Fig. 9. Two cells, the one to the right showing the acrosome carrier (K) far removed from the mitochondrial nebenkern and centriole. The cell to the left shows the nebenkern, part of the proximal centriole (C1), and the distal centriole (C2) at the edge of the cell. The distance between C1 and C2 becomes the region between C1 and C2 in Fig. 14. Compare this with figure 11 (C) for definitive position of proximal centriole with reference to the mitochondrial nebenkern. Approximately X 32,000.

Fig. 10. The spermatid nucleus has considerably elongated by this stage. Three acrosome carriers have been cut across, one attached to its cell. Compare this with Fig. 16. In the carrier in Fig. 10, what may be part of the flagellum appears to have become caught up in the carrier. Presumably the carrier pulls away from the tail later. Compare with Fig. 20 for absence of flagellum or centrioles in the carriers at the anterior end of the nearly ripe spermatozoa. Approximately X 32,000.

Fig. 11. The peculiar condition of the nuclear membrane is shown here. The proximal centriole is clear (C). The nebenkern with two mitochondrial profiles visible is now acquiring its shape of a reversed pyramid. Approximately X 32,000.
(Gatenby and Dalton: *Lumbricus* spermiogenesis)
Fig. 12. A good example of the junction (J) between cell and central nutrient mass (Y) which is non-nucleate, but contains yolk bodies and mitochondria. At the junction, a collar of slightly electron opaque fuzzy material is seen at F. The postnuclear (centriole adjunct) material (P) is clearly seen between two mitochondria. At C, there is a Y-shaped dense area marking the origin of the acrosome carrier. At T the material destined for inclusion in the middle-piece is seen. Approximately × 32,000.

Fig. 13. The nucleus has elongated considerably. The posterior end is above the letter M. In none of the micrographs did the junction between the nucleus and the mitochondrial nebenkern pass across at right angles to the length of the spermatid, an oblique junction being usual. Above and to the left of the letter N, a lower part of another tail filament is shown. At C₁ and C₂ there are the proximal and distal centrioles—no other centriole being found at the nuclear, middle-piece junction. The remarkable difference in the sizes of the middle-piece (M) and the nucleus is shown, though not all of the latter appears in this micrograph. Approximately × 13,500.

Fig. 14. Higher magnification of a portion of Fig. 13 showing detail of the apposition of nucleus (N) and middle-piece (M) just to the right of the left hand arrow. Detail of the central filament between C₁ and C₂ is also shown. Approximately × 32,000.
(Gatenby and Dalton: *Lumbricus* spermogenesis)
FIG. 15. Proximal lower (C) and probable distal upper (C) centrioles at the beginning of acrosome formation. At one period the proximal centriole approaches the nucleus (see also Fig. 1) during which the centriole adjunct or postnuclear peg is placed in situ. Later it withdraws from the peg, and becomes placed as in Fig. 11. For confirmation of this, see Figs. 13 and 14 (C₁, C₂). At T is the anlage of the material which later is incorporated into the nebenkern (see Fig. 17, T). Approximately × 32,000.

FIG. 16. The acrosome carrier (K) is now ready to receive the acrosome (A), which has left the acroblast (G). The carrier consists of three parts, a vacuole on the left, a granular mass, and the reception tube into which the acrosome slips. Compare both the acrosome carrier and nebenkern with those in Fig. 17. Note that the mitochondrial nebenkern is cut in approximate cross-section and consists of no more than six mitochondrial profiles. Approximately × 32,000.

FIG. 17. In the cell to the right, the mitochondrial nebenkern is cut in a plane perpendicular to that in Fig. 16, and exhibits but two mitochondrial profiles. Note the peg (P) and the material at its base shown also in Fig. 16. The cell to the left shows the reception tube for the acrosome which is either not visible or is incompletely formed. Compare with Fig. 16 and with Fig. 6 C where the tube is also forming. Approximately × 43,000.

FIG. 18. The acrosome has just been formed in connection with the Golgi apparatus (acroblast, dictyosome) (G). The mitochondrial nebenkern is partly cut at M. Approximately × 32,000.
(Gatenby and Dalton: *Lumbricus* spermiogenesis)
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Fig. 19. The anterior end of a nearly ripe spermatozoon. The acrosome has just become fixed. Approximately $\times 43,000$.

Fig. 20. The acrosomes have become elongated. The carriers, devoid of the acrosomes, are at K. Approximately $\times 32,000$.

Fig. 21. The postnuclear pin (P) is seen sticking into the nucleus. Approximately $\times 32,000$.
(Gatenby and Dalton: *Lumbricus* spermiogenesis)