The Spermatid Nucleus in Two Species of Grasshopper*

By JOSEPH G. GALL, PH.D., AND LOIS B. BJORK

(From the Department of Zoology, University of Minnesota, Minneapolis)

PLATES 230 TO 235

(Received for publication, January 14, 1958)

ABSTRACT

The nuclear changes accompanying spermatid elongation have been studied in two species of grasshopper, *Dissosteira carolina* and *Melanoplus femur-rubrum*. Testes were fixed in 1 per cent buffered OsO_4, imbedded in butyl methacrylate, and examined as thin sections in the electron microscope.

In both species nuclear changes during spermatid development involve (1) an early period, during which the nuclear contents are predominately fibrous; (2) a middle period, characterized by the lateral association of the nuclear fibers to form plates or lamellae which are oriented longitudinally in the major axis of the elongated nucleus; and (3) a late period, involving coalescence of the lamellae into a crystalline body which eventually becomes so dense that all resolvable detail is lost.

The fibers seen in the early spermatid nucleus are about 150 A in diameter and so are similar to fibers described from other types of nuclei. The thickness of the lamellae varies from about 150 A when first formed to 70 A during the later stages. The lack of evident chromosomal boundaries in the spermatid nucleus makes it difficult to relate either the fibers or lamellae to more familiar aspects of chromosome structure. We see no apparent reason to consider that the fiber alignment described here is related to conventional chromosome pairing.

Cytologists have long been interested in sperm development for a variety of reasons. The cytoplasmic phenomena following meiosis in the male germ cells are often of a spectacular nature, involving profound changes in all cell organelles. These include the condensation of the mitochondria into a compact mass known as the nebenkern, changes in the Golgi material leading to the formation of the acrosome, and the spinning out of the flagellum from one of the centrioles.

The nuclear changes are no less profound, although their true extent has only recently become apparent from electron microscopical observations. Earlier polarization studies on living material showed that many elongated sperm heads are negatively birefringent with respect to their major axis (13, 18). It was shown in some cases that a strong negative intrinsic birefringence is superimposed on a weaker positive form birefringence. Data from x-ray diffraction experiments on sperm of *Sepia*, *Loligo*, and *Salmo* show a crystalline arrangement of at least some of the DNA present in the head (20). The polarization optical and x-ray studies together indicate that in such elongated sperm heads the DNA molecules are aligned parallel to the long axis of the nucleus. Finally, marked changes in the staining capacity of the nucleus and its response to enzymatic digestion often occur during spermatid development when the molecular orientation is taking place (4, 6).

Electron microscopical observations have recently confirmed the existence of highly oriented nuclear material during spermatid development in several species of snail (9, 14) and grasshopper (8, 22). In the cases cited, the elongated nucleus contains numerous plate-like structures arranged parallel to its long axis. In later stages the plates seem to fuse into a honeycomb arrangement or labyrinthine pattern, which becomes so dense in

* Supported by funds from the National Science Foundation and the Graduate School of the University of Minnesota.

the mature sperm head that no fine structure is resolvable. Not all animal species show the type of plate-like orientation just mentioned. For instance, the spermatid nuclei of the cat (2) and toad (3) contain numerous very dense granules 600 to 700 A in diameter.

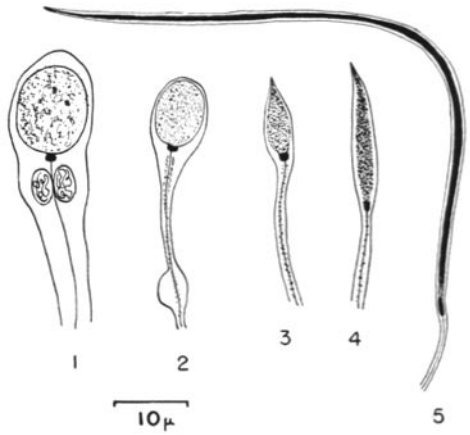
The present observations extend those of Gibbons and Bradfield (8) and Yasuzumi and Ishida (22) to two additional species of grasshopper. Evidence is also presented for the origin of plate-like structures from the fibrous nuclear contents of the early spermatid.

Materials and Methods

We have studied the two species of grasshopper, *Dissosteira carolina* and *Melanoplus femur-rubrum*. The abdomen of each animal was opened and the testes quickly located, using the low power of a binocular dissecting microscope. If the testis is grasped with one pair of jewelers' forceps at the point where the follicles are joined together, the interfollicular fat can usually be removed by a raking motion with a second pair of forceps. Fixation was carried out in buffered 1 per cent osmium tetroxide according to Palade (12) or Sjöstrand (19); spermatids seemed equally well fixed by the two solutions. Fixation times were short, usually from 30 minutes to 4 hours at the most. Washing in water and 70 per cent ethanol was brief, about 15 minutes in each, followed by several changes of 95 per cent and 100 per cent ethanol extending over several hours. All the material was imbedded in butyl methacrylate to which was added 1 per cent benzoyl peroxide as catalyst. Polymerization was carried out in an oven at 45° or 60°C., or under a small germicidal UV lamp. Thin sections were cut with glass knives using the Porter-Blum microtome, and examined without removal of the plastic in the RCA electron microscope EMU-2 at initial magnifications from 2,000 to 15,000.

OBSERVATIONS

The gross changes in the grasshopper spermatid during its period of elongation into the mature sperm are well known from studies made with the light microscope. For purposes of orientation the general form changes are shown in Text-fig. 1, drawn from observations on both fixed and living material. During the whole of spermatid development, the originally round nucleus undergoes a progressive thinning and elongation until it forms the thread-like head of the mature sperm. The mature sperm nucleus of *Melanoplus* is 125 μ in length, but less than 0.3 μ in width. Surrounding the head at all stages is a thin layer of cytoplasm,



TEXT-FIG. 1. Sketches to show the general form changes involved in the transformation of a spermatid into a mature sperm. Only the nucleus is accurately depicted. Drawn from both living and fixed spermatids of *Dissosteira*. In *1* can be seen the nearly spherical nucleus, the centriole and its associated flagellum, and the two halves of the nebenkern. The most striking changes in *2* through *5* are the extreme thinning and elongation of the nucleus and the pulling out of the halves of the nebenkern to form a sheath around the flagellum.

and the posterior boundary of the nucleus is marked by a prominent centriole. Very little internal nuclear detail is visible with the light microscope during the course of spermatid elongation.

The electron microscope shows a wealth of detail, however. The most striking feature is the presence of oriented plates or lamellae aligned parallel to the long axis of the spermatid nucleus. For descriptive purposes, we can divide the sequence of nuclear changes into three overlapping periods: an *early* stage during which the nucleus contains numerous very fine fibers; a *middle* period characterized by the formation of lamellae, apparently by the lateral association of the original fibers; and a *late* stage during which the lamellae are converted into a rough honeycomb pattern which finally approaches a crystalline arrangement. In the mature sperm no internal nuclear detail has been resolved either in this or other studies dealing with the grasshopper.[1]

[1] Dass and Ris, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 129, have recently described a similar sequence of events during spermatid development in several species of grasshopper.

Early Period.—From the telophase of the second meiotic division until after the beginning of spermatid elongation, sections of the nucleus display both linear and punctate profiles about 150 A in diameter. Such an appearance could be produced by a nucleus that in life contained only a mass of long coiled threads, the dot-like profiles being merely cross-sections of the threads. On the other hand, some of the dots could as well be sections of granules. At present a choice between the two interpretations is not possible, nor can we say anything about the length of the fibers, since the section thickness does not permit following individual fibers for more than a few hundred angstroms.

After the nucleus has started its elongation, one can say with some assurance that its contents are largely fibrous. The evidence is that longitudinal sections of the nucleus show predominantly short fibers, whereas sections perpendicular to the long axis show many more dots. In other words, there has been a rough alignment of the fibrous nuclear contents in such a manner that the long axis of many fibers lies parallel to the long axis of the nucleus (Figs. 1 and 2). This orientation is most striking at the basal end of the nucleus next to the centriole. Fig. 6, for instance, shows the basal portion of an elongating spermatid nucleus of *Melanoplus* with the adjacent centriole; here one sees a predominantly longitudinal orientation of fibers. Fig. 7 is an oblique section of a comparable spermatid in which one finds not only short linear profiles, but also the expected dot-like cross-sections of many of the fibers. An oblique section was purposely chosen for illustration, since the inclusion of a portion of the centriole proves we are dealing with the same part of the nucleus that was shown in Fig. 6. Further evidence for the fibrous, rather than lamellar, nature of the profiles seen in Figs. 1 and 6 is their relatively uniform thickness and short length. As will be shown later, longitudinal sections of lamellae can often be followed for distances of several micra, and their profiles will be of uniform thickness only when very highly oriented. We wish to stress the existence of fibers during the early stage of spermatid differentiation, because the peculiar lamellated structure of the more mature nucleus must somehow be derived from them.

A closer examination of Fig. 1 is instructive in suggesting a mode of origin for the lamellae which will subsequently fill the nucleus. One sees here numerous short segments of fibers arranged for the most part in the long axis of the nucleus. In many places groups of fibers are lined up side by side and are separated from similar groups by less dense areas that contain few or no fibers (Fig. 1, inset). This general picture suggests that the fibers are beginning to associate laterally into plates, somewhat as the fingers do in one's hands; the lighter areas represent the spaces between successive lamellae. This interpretation of the early stages becomes considerably more cogent in light of the changes occurring next.

Middle Period.—The middle period of spermatid development, as here defined, includes the transformation of the predominately fibrous contents of the early nucleus into lamellae or plates, and the gradual condensation of these plates into a honeycomb arrangement. Micrographs of both longitudinal and cross-sections of these stages are shown in Figs. 3 to 10. Similar micrographs have been presented by Gibbons and Bradfield (8) working with *Locusta*, and by Yasuzumi and Ishida (22), who picture the later stages in *Gelastorhinus*. What are probably essentially similar structures, though differing in some detail, are described by Grassé *et al.* (9) and by Rebhun (14), who studied spermatid development in species of snails. Of the authors referred to, all but Grassé *et al.* agree that the nucleus at this stage contains numerous lamellar structures. Grassé *et al.*, on the other hand, interpret very similar pictures as showing thin fibrous chromonemata. The middle stages in *Dissosteira* and *Melanoplus* must, however, be largely sheet-like for the following reasons. Longitudinal sections show predominately linear profiles (Figs. 3, 5, and 8), while transverse sections show broadly waving lines or a labyrinthine pattern (Figs. 4 and 9). Only near the centriole or in cross-sections of early stages (Fig. 2) does one see the dot-like sections indicative of fibers. Thus the images met with in middle stage spermatids are most easily interpreted as sections through curtain-like structures which extend parallel to the major axis of the sperm head.

Although the general features are relatively clear, some of the finer points of morphology can be determined only by more extensive study. For instance, we cannot be sure of the maximum length of the plates, although many can be followed for at least 2 or 3 micra in the longitudinal axis of the nucleus (Fig. 8). Likewise, without an exhaustive study of serial sections, we cannot

determine whether all the lamellae eventually unite with the nuclear membrane, as suggested by Rebhun (14).

Further evidence for the lamellar nature of the profiles is found in longitudinal sections, such as Fig. 8. Here one can see not only sharp, dense lines, where the lamellae are cut perpendicular to their faces, but also more indistinct lines and diffuse areas of medium density. The latter are obviously oblique sections and face-on views of the lamellae which are expected from the wavy contours of the profiles seen in the cross-sections (Fig. 9). By contrast, only sharp lines are found in the transverse sections (Figs. 4, 9, and 10), indicating that the lamellae are strongly oriented in the longitudinal axis of the sperm head. Another feature evident from cross-sections such as Fig. 4 is the variable length of the lamellar profiles in this plane; some are long, sinuous lines indicating lamellae of 1 or 2 micra width, while others are only a few hundred angstroms wide. Some of the profiles describe closed figures, and frequent branches occur. Both of these latter features become more common in the older spermatid nuclei (Figs. 9 and 10) as the nuclear material becomes more compact.

During the middle period of spermatid development, the thickness of the plates shows a progressive decrease from a maximum of about 150 A to approximately 70 A. The thickness can be most easily measured from transverse sections of the nucleus where most of the plates are cut perpendicular to their flat faces; similar values are obtained by taking the minimal width of the profiles seen in longitudinal section. Actually, the earlier plates are difficult to measure accurately because of their indefinite borders. The same difficulty was noted by Gibbons and Bradfield (8), whose figure of 60 to 100 A refers only to the denser central portion of the lamellar profile. The minimal thickness which we have measured agrees well with that given by Gibbons and Bradfield and also with the figures quoted by Rebhun and Grassé *et al.* for the snail (60 A and 70 to 80 A, respectively).

Late Period.—The final stages of nuclear transformation involve a progressive packing of the lamellae as the nucleus still further elongates. The sinuous profiles seen in Fig. 9 are first converted into a loose honeycomb arrangement, as in Fig. 10, and eventually take on a very regular arrangement. The very latest stages in which resolvable detail is present display a remarkable crystalline appearance in transverse section (Fig. 11). The high degree of orientation within the nucleus, long known from polarization optical and x-ray analysis, is thus amply confirmed by electron microscopic observations.

Eventually, the nucleus undergoes still further elongation, accompanied by an increase in density and the disappearance of all resolvable detail (Fig. 12). Since the fully developed sperm head shows a strong birefringence (13), the loss of resolvable detail cannot be ascribed to a loss of orientation, but probably involves further condensation.

DISCUSSION

The contents of the spermatid nucleus during the *middle* stage of differentiation are clearly in the form of curtain-like lamellae oriented in the long axis of the sperm head. This interpretation, which supports the findings of Rebhun (14) and Gibbons and Bradfield (8), differs from that of Grassé *et al.* (9), who describe thread-like chromonemata in the spermatid nucleus of snails. We wish to emphasize that this difference is essentially one of interpretation, since the pictures of Grassé *et al.* are very similar to those of Rebhun and not unlike some presented here. The evidence for sheets or lamellae rests on two main arguments: (*a*) Longitudinal and transverse sections of the same stage show linear profiles, in the first instance following straight paths parallel to the axis of the elongated nucleus, in the second following a meandering course. Threads should show predominately dots in transverse section. Grassé *et al.* do present a single picture of a nucleus filled with dots, but Rebhun claims he has never seen similar cross-sections in his study of the snail. (*b*) The lines seen in longitudinal sections have a *minimal* width of 70 to 150 A, depending upon the stage of development. Many are much wider, however, as expected for lamellae sectioned obliquely to their plane surface. Threads oriented in the long axis of the spermatid nucleus would have a *maximal* profile width equal to their full diameter. If this diameter is less than the section thickness, as would be the case for threads less than 150 A diameter, the profiles should be of very uniform appearance.

Granting that plate-like structures are present during the middle stage of development, we may ask about their origin. Our belief is that they are

formed by the lateral association of the fine threads seen during the early period of spermatid elongation, and that these fine threads are essentially similar to those found in many other types of nuclei. The main lines of evidence have already been presented, but may be summarized briefly as follows: (*a*) Preceding the formation of the lamellae there is a stage during which fibers are present and are roughly oriented into plate-like bundles in the long axis of the nucleus (Fig. 1). (*b*) The fibrous condition is very marked at the centriole end of the nucleus, even during the middle stage of development (Figs. 6 and 7), and a transitional zone between fibers and plates is found near the base of the nucleus. (*c*) The fiber diameter in the early spermatid is approximately equal to the lamellar thickness (150 A).

We are thus in agreement with Grassé *et al.* in describing the original orientation of nuclear materials in the spermatid as involving fibers. Our study, like theirs, places emphasis on the centriole as a center of this organization. Rebhun also suggests an origin of the later lamellae from the lateral association of fibers in the early spermatid, but does not stress the point. The only essential disagreement among the various studies cited, therefore, seems to be the failure of Grassé *et al.* to recognize the lamellar nature of the structures seen during the middle period of development.

Any attempt to correlate the fiber structure seen during the early stages of spermatid development with chromosome structure in general is hampered by our poor understanding of the latter subject. Most accounts ascribe a predominately fibrous fine structure to chromosomes, but the details as to number of fibers or their diameters differ considerably (*e.g.* 1, 5, 7, 10, 11, 15, 21, 23). It is perhaps safe to say that the majority of workers believe the smallest fiber units of chromosomes (whether called microfibrils, chromofibrils, chromonemata, etc.) are of the order of 100 to 500 A in diameter, but micra in length. The differences in actual figures quoted may be due in part to the different types of chromosomes studied and to differences in preparative techniques. Whether high resolution studies will eventually identify a single basic thread of relatively constant dimensions we cannot say at the present.

The formation of lamellae from the chromosomal fibers of the early stage raises interesting questions about the nature of the association between the fibers. As interpreted here, the fibers align side by side somewhat like the fingers of one's hand. This process seemingly implies a bilateral symmetry of the fibers such that a limited number of pairing groups is available for association with adjacent fibers. Otherwise one might expect to find fiber association leading to the formation of thick bundles, rather than flat plates.

Whatever the nature of the fiber association, we see no *a priori* reason to equate it with the well known pairing of homologous chromosomes which occurs earlier in meiosis; or to be more general, with any scheme based on genetic similarity of the fibers (since the spermatid of course contains only a haploid set of chromosomes). To arrive at this conclusion, we have considered the two theoretical possibilities that (*a*) a chromosome consists of only a small number of long submicroscopic strands, or (*b*) it is composed of a large number (say, 10's or 100's) of identical strands. In the first case, a lamella could only be formed by a complex folding of the few strands of one chromosome or by the association of regions from several chromosomes. This fact follows directly from the observed widths of the lamellae which are certainly of the order of micra in the middle stage (Fig. 4). In either case, non-homologous portions of strands would have to be associated. On the other hand, if a chromosome is multistranded and the association "gene for gene," then we might perhaps expect to find the number of lamellae in some fashion related to the number of chromosomes (11 or 12 in the present cases). This is not a rigorous logical necessity, however; for instance, all the chromosomes might be lined up end to end in the spermatid head, so that a cross-section at any one level would pass through only a single chromosome and its constituent lamellae. But we can at least say that the observed arrangement of lamellae gives no suggestion of chromosome boundaries, and, in the later stages of transformation, all the nuclear material in any transverse section forms a fairly homogeneous crystalline body.

It is beyond the scope of this paper to enter a discussion of the evidence for and against multistrandedness of chromosomes. The evidence from several sources (crossing over, breakage by ionizing radiations, and the distribution of radioactivity in the daughter strands of isotopically labelled chromosomes) has been variously interpreted, and it seems premature to assume, as some have, that all chromosomes consist of very large numbers of

similar or identical units. Unfortunately, the electron microscopic observations on spermatids do not supply decisive evidence at present. We have tentatively concluded that, regardless of the number of strands per chromosome, the alignment of fibers into plates is not readily explicable in terms of "conventional" chromosome pairing.

We have stated earlier that the well known anisotropic nature of the grasshopper spermatid and sperm as seen under the polarizing microscope (13) correlates nicely with the high degree of orientation seen in the electron microscope. A word of caution is necessary, however, in assuming that all anisotropic sperm will show similar plate-like formations. Pattri (13) states that the elongated sperm head of the toad *Bombinator* is, like other elongated sperm heads birefringent. However, the study of Burgos and Fawcett (3) on the similarly elongated head of *Bufo* fails to show any sign of lamellae. Likewise the sperm head of the salamander, *Triton* (=*Triturus*) *taeniatus* is birefringent (18), but some preliminary observations on *Triturus viridescens* have failed to demonstrate lamellae in the spermatids. Clearly some careful comparative studies are necessary before we can say with certainty what aspect of the electron micrographs really correlates with the birefringence data. It is possible that the DNA molecules show a high degree of longitudinal organization leading to birefringence without a corresponding organization at the somewhat grosser level described here.

Finally, the crystalline arrangement of nucleoprotein demonstrated in the later spermatids (Fig. 11) is not peculiar to sperm heads. A quite similar picture was recently demonstrated in the macronucleus of the protozoon *Tokophrya* (17). We may, perhaps expect to find a somewhat similar degree of crystallinity in still other cases where the nuclear contents are highly concentrated.

Bibliography

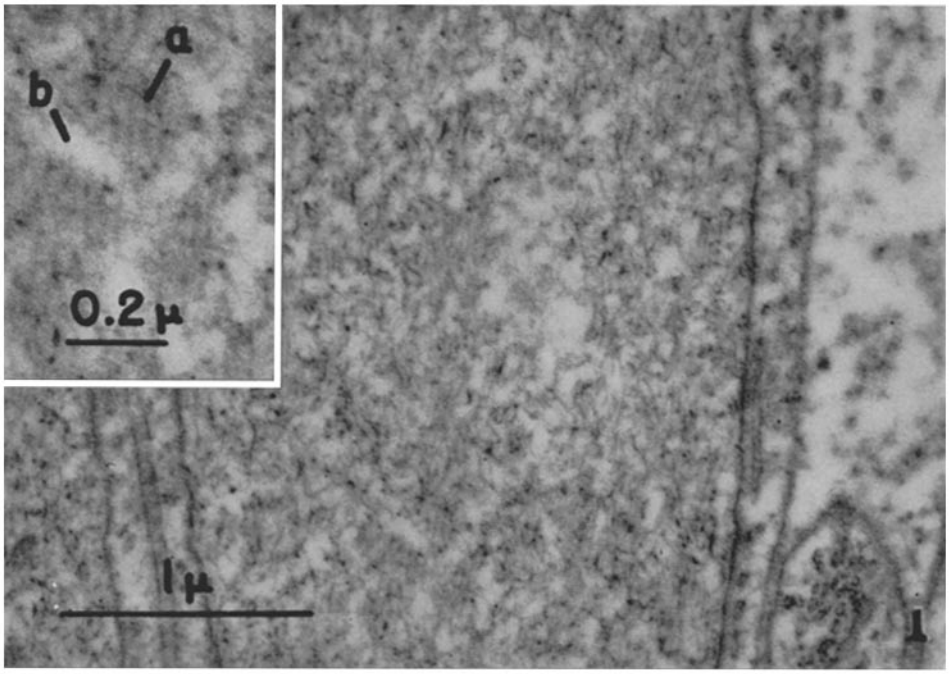
1. Beermann, W., and Bahr, G. F., *Exp. Cell Research*, 1954, **6,** 195.
2. Burgos, M. H., and Fawcett, D. W., *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** 287.
3. Burgos, M. H., and Fawcett, D. W., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 223.
4. Daoust, R., and Clermont, Y., *Am. J. Anat.*, 1955, **96,** 255.
5. Gall, J. G., *Brookhaven Symp. Biol.*, 1955, **8,** 17.
6. Gatenby, J. B., *Quart. J. Micr. Sc.*, 1918, **63,** 197.
7. Gay, H., *Proc. Nat. Acad. Sc.*, 1955, **41,** 370.
8. Gibbons, I. R., and Bradfield, J. R. G., *J. Biophysic. and Biochem. Cytol.*, 1957, **3,** 133.
9. Grassé, P. P., Carasso, N., and Favard, P., *Ann. sc. nat. zool.*, 1956, **18,** 340.
10. Kaufmann, B. P., and McDonald, M. R., *Cold Spring Harbor Symp. Quant. Biol.*, 1956, **21,** 233.
11. Nebel, B., *J. Hered.*, 1957, **48,** 51.
12. Palade, G. E., *J. Exp. Med.*, 1952, **95,** 295.
13. Pattri, H. D. E., *Z. Zellforsch. u. mikr. Anat.*, 1932, **16,** 723.
14. Rebhun, L. I., *J. Biophysic. and Biochem. Cytol.*, 1957, **3,** 509.
15. Ris, H., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** No. 4, suppl., 385.
16. Roth, L. E., *J. Biophysic. and Biochem. Cytol.*, 1957, **3,** 816.
17. Rudzinska, M. A., and Porter, K. R., *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** 421.
18. Schmidt, W. J., Die Doppelbrechung von Karyoplasma, Zytoplasma und Metaplasma, Protoplasma Monographien, Berlin, Borntraeger, **11,** 1937.
19. Sjöstrand, F., *in* Physical Techniques in Biological Research, (G. Oster and A. W. Pollister, editors), 1956, **3,** chapter 6.
20. Wilkins, M. H. F., and Randall, J. T., *Biochim. et Biophysica Acta*, 1953, **10,** 192.
21. Yasuzumi, G., *Exp. Cell Research*, 1956, **11,** 240.
22. Yasuzumi, G., and Ishida, H., *J. Biophysic. and Biochem. Cytol.*, 1957, **3,** 663.
23. Yasuzumi, G., Odate, Z., and Ota, Y., *Cytologia*, 1951, **16,** 233.

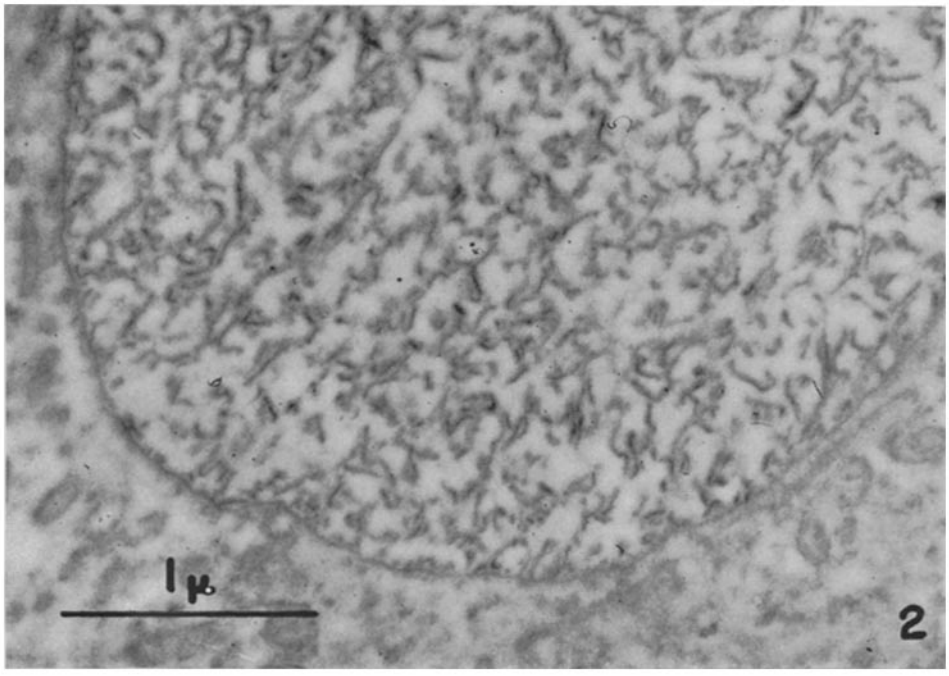
Explanation of Plates

Plate 230

Fig. 1. Spermatid of *Melanoplus* in longitudinal section, showing an early stage in the orientation of the chromosomal fibrils. As seen more clearly in the inset, small groups of fibrils each about 150 A diameter, tend to be aligned in areas of greater density. These areas of fiber orientation (*a*) are separated from one another by areas of lower density (*b*). The darker areas are interpreted as regions where the chromosomal fibrils are aggregating to form lamellae. × 32,500. Inset, × 65,000.

Fig. 2. *Dissosteira* spermatid nucleus in transverse section. The stage illustrated here is somewhat more advanced than that in Fig. 1. One can see dots which are probably transverse sections of fibrils, and short linear profiles which are sections of small lamellae or short segments of fibrils (see text). The thickness of the linear profiles is somewhat difficult to measure due to their diffuse outline, but averages about 150 A. × 33,000.





(Gall and Bjork: Grasshopper spermatid nucleus)

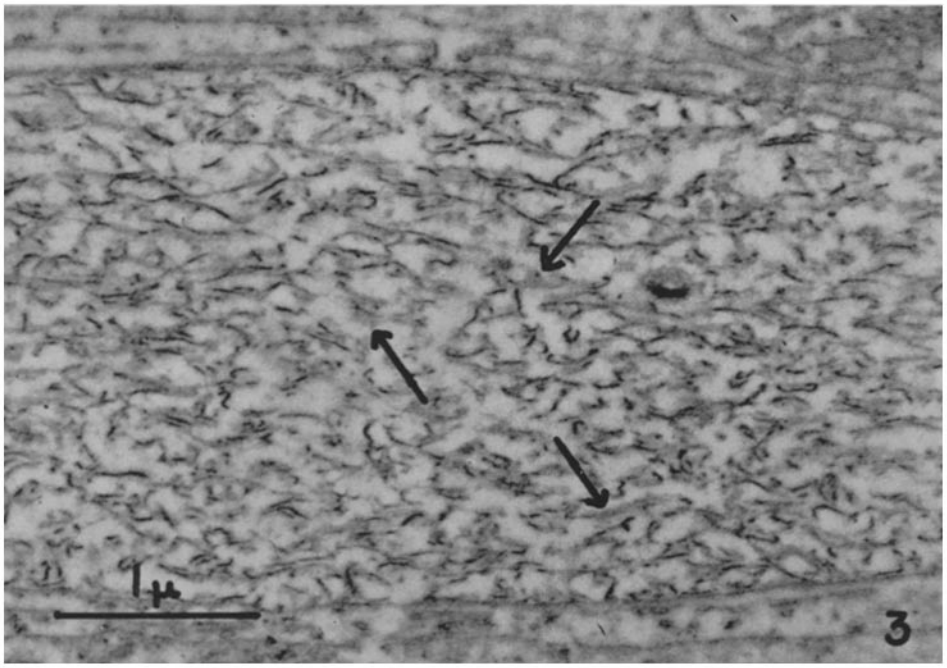
PLATE 231

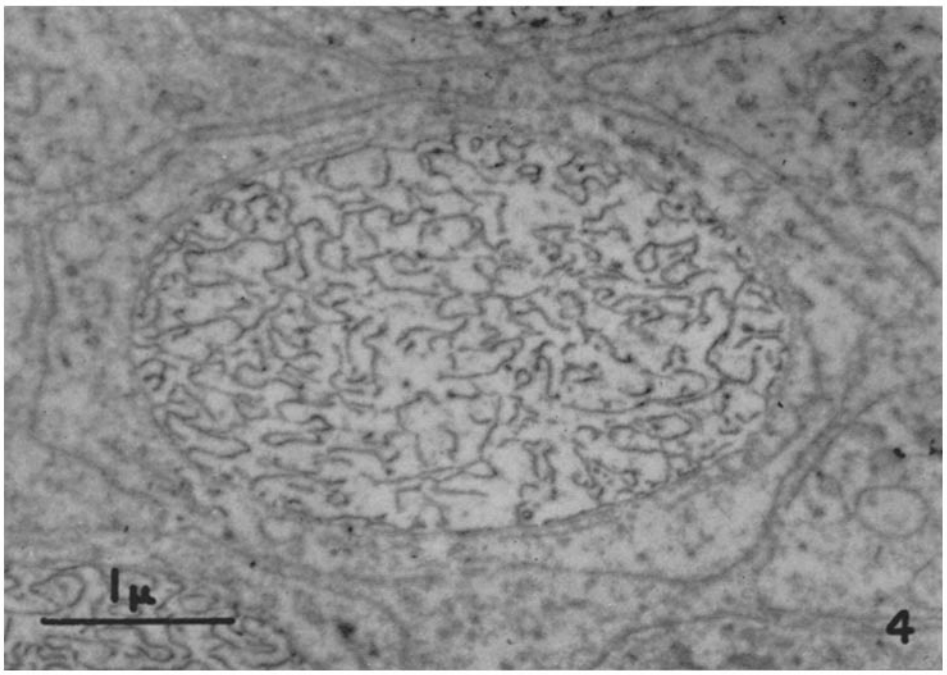
FIGS. 3 and 4. *Dissosteira* spermatid nuclei of comparable age in longitudinal and transverse section respectively. By this stage, the nucleus contains numerous plate-like structures with an increasing longitudinal orientation. The plate thickness is about 130 A.

Note in Fig. 3 (arrows) that many of the profiles are indistinct or fade into wider gray areas; such regions are interpreted as full faced views of the plates, and would not be found in longitudinal sections through bundles of fibers (*cf.* Figs. 6 and 7).

In Fig. 4 the sinuous profiles are for the most part of rather sharp outline. This fact indicates that the lamellae are cut quite transversely and are therefore strongly oriented in the long axis of the nucleus.

Fig. 3, × 27,000. Fig. 4, × 25,000.





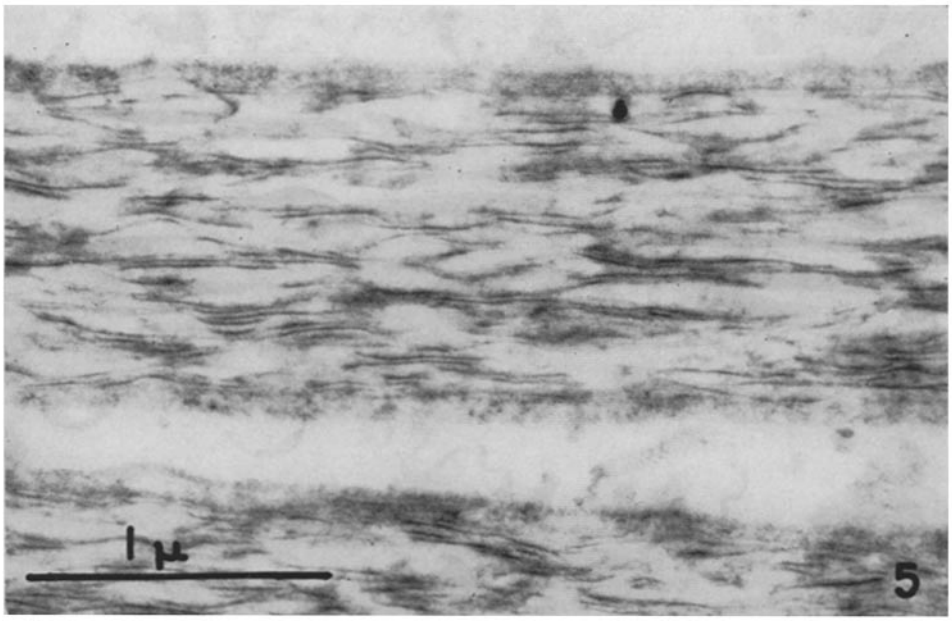
(Gall and Bjork: Grasshopper spermatid nucleus)

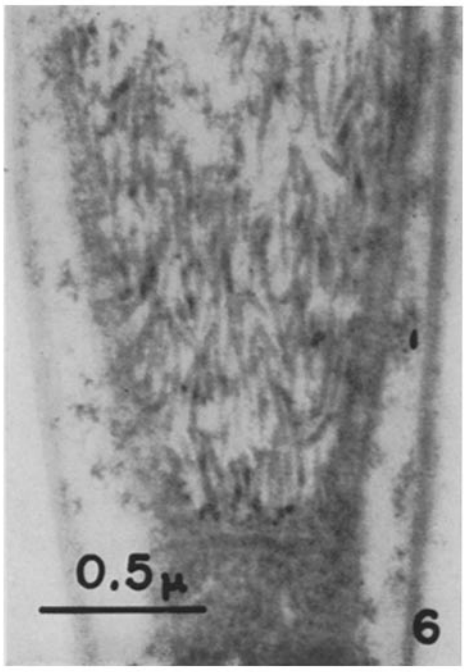
PLATE 232

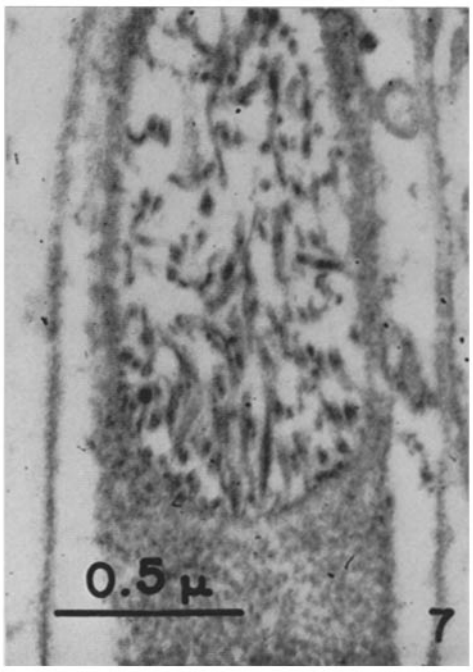
FIG. 5. Longitudinal section of *Dissosteira* spermatid in the middle stage of development. This somewhat thicker section is included to demonstrate the obvious lamellar nature of the denser parts of the nucleus. Not only does one see relatively sharp profiles, corresponding to lamellae cut transversely, but also more diffuse areas cut obliquely to the lamellar surfaces. Lamellar thickness about 100 A. × 41,000.

FIG. 6. The basal or centriole end of a spermatid nucleus of *Dissosteira*, sectioned longitudinally. This nucleus is in approximately the same stage as that shown in Fig. 5, as judged by regions anterior to that shown here. Curiously, however, the material near the centriole maintains its fibrous character long after most of the nuclear contents are distinctly lamellar. That we are dealing with fibers is indicated by three facts: (*a*) the relatively uniform thickness (150 to 200 A) of the profiles, (*b*) their short length in the plane of the section, and (*c*) the dot-like appearance of their cross-sections (Fig. 7). The first two facts are not sufficient criteria in themselves, but are expected for fibers whose diameter is less than the section thickness. Lamellae must be highly oriented, as in parts of Fig. 8, to show profiles of uniform thickness, and then can be followed for long distances in the plane of the section. × 44,000.

FIG. 7. Same stage, sectioned obliquely to the spermatid axis. The presence of the centriole (granular material at the bottom) shows we are dealing with the same part of the nucleus that is shown in Fig. 6. This micrograph demonstrates dot-like cross-sections of the fibers present in the basal part of the spermatid nucleus. Other micrographs, not reproduced here, suggest that these fibers may in fact be fine tubules. × 47,000.



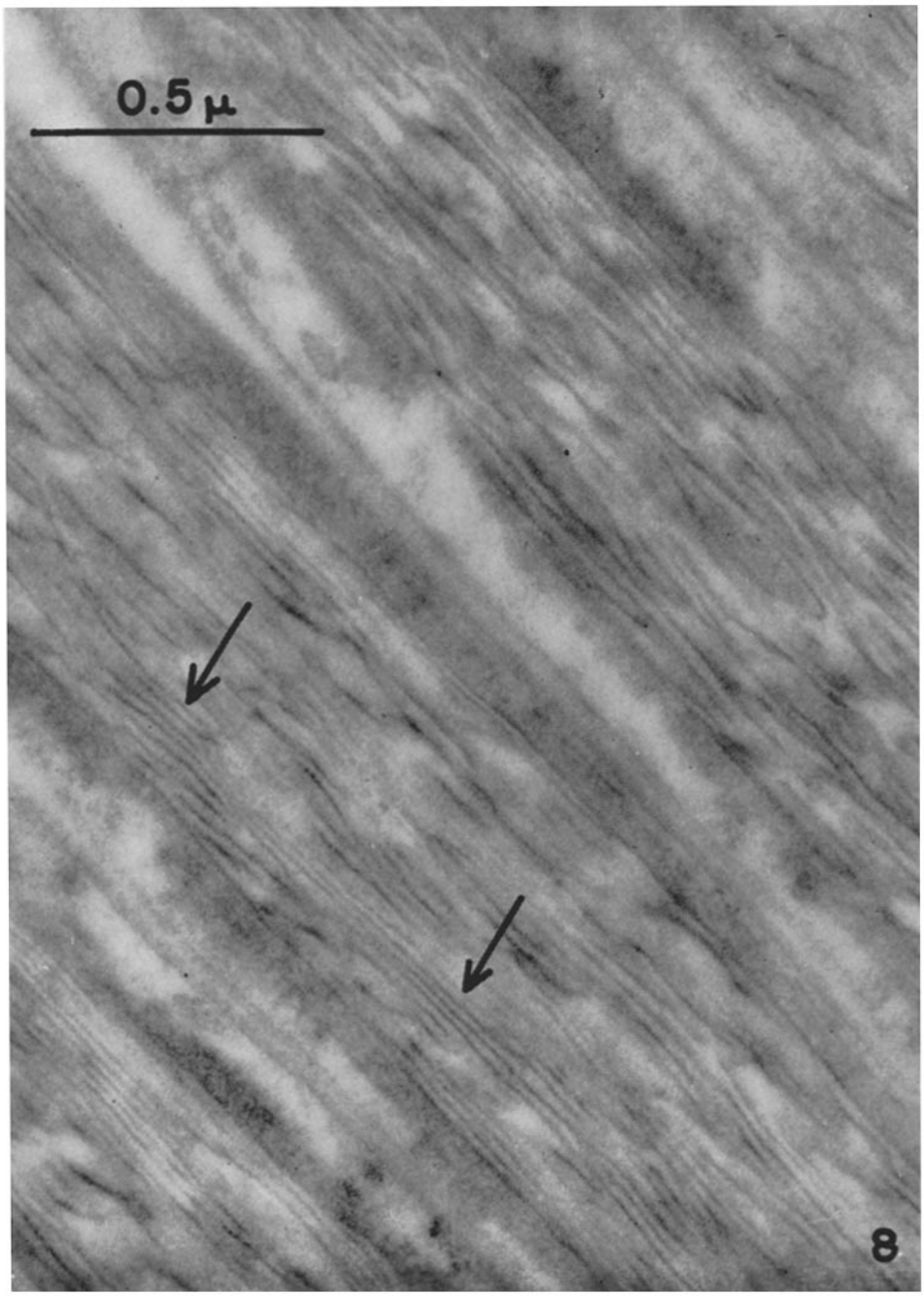




(Gall and Bjork: Grasshopper spermatid nucleus)

PLATE 233

FIG. 8. Longitudinal section of several elongated spermatid nuclei of *Melanoplus*, showing the increasing orientation of the lamellae. Because of the varying planes of sectioning within the same nucleus, the lamellae show a variety of apparent thicknesses; the minimal value seen is about 80 A (arrows), and is thus closest to the true thickness of the plates. The distance between plates is a variable depending upon the stage of differentiation of the nucleus; furthermore, not until later stages does the packing within any one nucleus become fairly regular (Fig. 11). × 81,000.

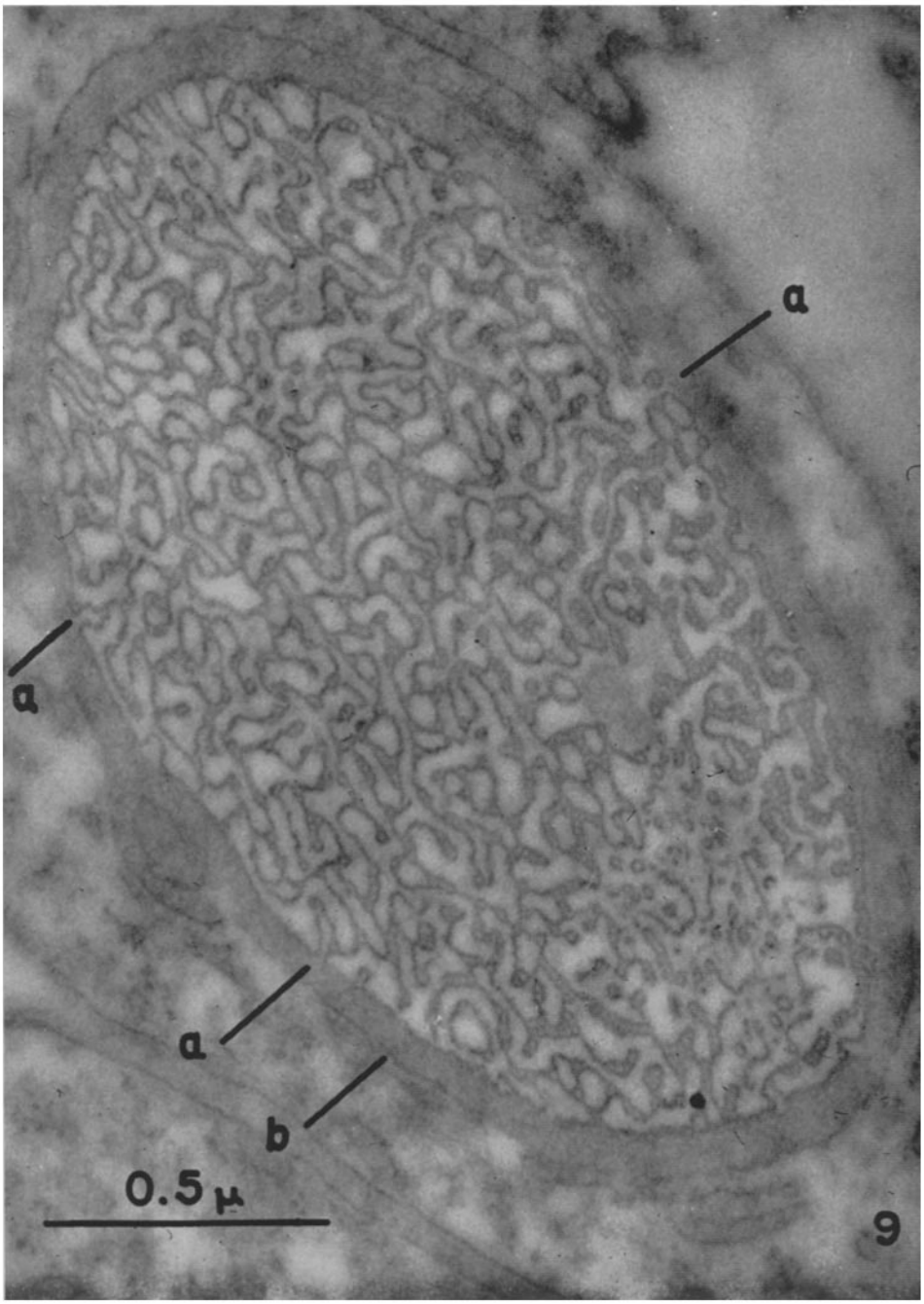


(Gall and Bjork: Grasshopper spermatid nucleus)

PLATE 234

FIG. 9. *Melanoplus* spermatid in slightly oblique section, showing the labyrinthine pattern assumed by the nuclear contents after further packing and coalescence of the lamellae. In many areas the material has a bubbly appearance, which seems to be a foreshadowing of the honeycomb packing which occurs next. How such an arrangement arises is not at all clear from the available pictures of this stage.

The nature of the nuclear envelope is quite unusual in this material. Apparent free access to the interior of the nucleus is possible at a number of points along the nuclear margin (*a*). However, the "holes" are of varying sizes and generally smaller than the regular discontinuities seen in somatic nuclear envelopes. Furthermore, the nuclear boundary, at least as seen in our photographs, seems to be a single membrane fully continuous with the internal lamellae. Perhaps the outer of the two membranes comprising the customary nuclear envelope is actually that shown at *b*. In this case, the wide dense area around the nucleus would be a swollen perinuclear cisterna. Closer study of the earlier stages will be needed to settle this question. × 77,000.



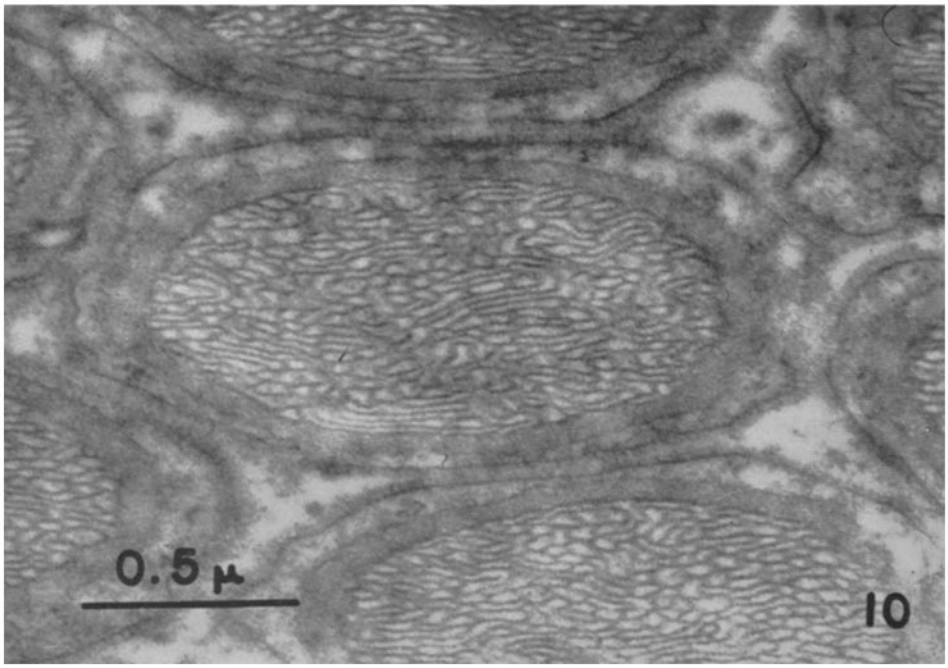
(Gall and Bjork: Grasshopper spermatid nucleus)

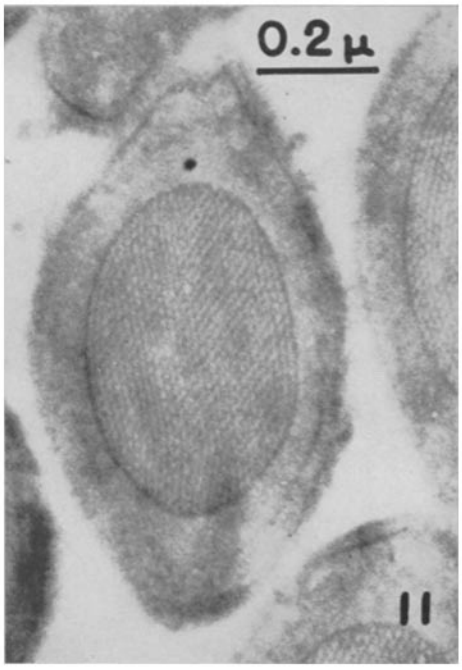
PLATE 235

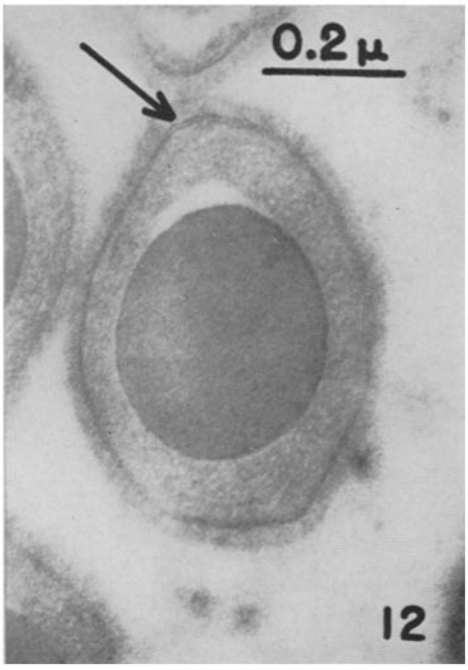
FIG. 10. Slightly oblique section of honeycomb stage in *Melanoplus* spermatid nucleus. The lamellae are now fused into a complex anastomosing system. × 59,000.

FIG. 11. Late spermatid of *Melanoplus*, showing the crystalline packing finally assumed by the nuclear contents. The parallel lines seen here have a regular periodic spacing of about 140 A. The lines themselves, which are presumably derived from the lamellae seen in earlier spermatids, are about 70 A thick. × 76,000.

FIG. 12. Very late spermatid of *Melanoplus* in which resolvable nuclear detail is no longer present. The surface of the spermatid consists of a pair of delicate membranes about 50 A thick (arrow) separated by a space of the same magnitude. Outside these membranes is a diffuse region with some indication of the radial structure recently described by Roth (16). × 98,000.







(Gall and Bjork: Grasshopper spermatid nucleus)