THE FINE STRUCTURE OF NUCLEI DURING SPERM MATURATION IN THE LOCUST

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Plates 23 to 25

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INTRODUCTION

In the course of a general investigation of methods of fixation of cell nuclei in locust testis, a preliminary account of which has been published elsewhere, we have encountered highly orientated structure in the chromatin of maturing sperm heads. This structure is of a kind which might be expected from the birefringence studies of Schmidt (14) and Pattri (12) and of the ultraviolet dichroism studies of Caspersson (2), which have produced evidence for the existence in locust sperm heads of a marked orientation of desoxyribonucleic acid molecules parallel with the long axis of the head.

Rather similar structures have been reported by Grassé, Carasso, and Favard (7–9) in other invertebrate sperm heads, though the detailed structure in locust differs from that reported by these authors.

Material and Methods

The procedure for fixing testis from the locust, *Locusta migratoria*, in buffered isotonic 1 per cent osmium tetroxide solution and for subsequent dehydration, embedding, and sectioning has been described previously (4). In these studies testes were also fixed in a 5 per cent solution of formaldehyde made up in acetate-veronal buffer at pH 7.3; and in 45 per cent acetic acid containing 0.9 per cent sodium chloride to prevent the cells bursting.

OBSERVATIONS

In the primary and secondary spermatocyte nuclei of the locust there is little easily distinguishable fine structure in the chromatin after osmium tetroxide fixation, although there have recently been reports of a tubular core to meiotic prophase chromosomes (11). There is a similar apparent lack of general organisation after formaldehyde or acetic acid fixation. In cells fixed with osmium tetroxide even the distribution of chromatin in the nucleus is difficult to distinguish—presumably because the uptake of osmium per unit of dry matter is greater in the nuclear sap than in chromatin so that the natural contrast be-

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tween the two is diminished and a false homogeneity appears (4). Certainly the natural contrast between chromatin and nuclear sap is much more conspicuous in cells fixed in formaldehyde or in acetic acid, where no heavy atoms have been introduced (6). It is, however, necessary with these fixatives to use rather thicker sections than with osmium tetroxide—unless suitable electron stains are used (5).

As the spermatid begins to elongate there appear in electron micrographs of sections through its nucleus conspicuous dark lines up to 0.2 μ long (Fig. 1). Most, but not all, of these dark lines run roughly parallel with the long axis of the sperm. The thickness of the most electron-dense parts of the dark lines varies between 60 A and 100 A, but there often occur irregular areas of less dense material on each side of the dark lines. As the spermatid becomes more mature the dark lines apparent in longitudinal sections of the nucleus become longer, more regular in width, and more closely parallel to the long axis. Fig. 5 shows a longitudinal section through a nucleus in an advanced stage of orientation. Fig. 3 shows a longitudinal section through the nucleus of a less mature spermatid, intermediate in degree of orientation between those shown in Fig. 1 and Fig. 5. In sections of the best orientated nuclei it is sometimes possible to trace the dark lines for 2 μ and they probably extend further than this. Their thickness is approximately 60 A; it is, of course, difficult to measure accurately owing to the difficulty of defining their edges. Their lateral spacing is less difficult to measure, but it appears in sections to vary, even within one nucleus, between 180 A and 240 A (centre to centre). This variability may be real or due to artefacts. The dark lines sometimes appear to join, but any judgement on this point is complicated by the possibility of overlay. In several places (*e.g.* the insert on Fig. 5) we have observed what appears to be a periodic structure along the lines (period about 75 A), but we hesitate to emphasize this feature until more work has been done.

So far we have referred only to *longitudinal* sections. *Transverse* sections of the best orientated nuclei show a mass of contiguous polygons of somewhat variable shape and size (Fig. 6); their dimensions have probably been somewhat distorted during sectioning. In this particular nucleus there are about 275 of these polygons, but other nuclei we have counted have contained numbers varying from 36 to 350—in general, the higher numbers coming from nuclei with relatively large cross-sectional area and *vice versa*. In correlating longitudinal and transverse sections it is possible to make use of a convenient feature of sperm maturation in locust testis—namely that all cells in any one cyst develop synchronously. Fig. 6 is a transverse section from the same cyst as produced the longitudinal section shown in Fig. 5 and hence these two must show the same stage of development. Similarly Figs. 3 and 4 are from the same cyst—a less mature cyst than that from which Figs. 5 and 6 were taken. Fig. 4

shows a mixture of complete and incomplete polygons together with various vague loops; it is an intermediate stage in the development of the more uniform and complete polygons shown in Fig. 6, (just as Fig. 3 shows an intermediate stage in the development of the condition shown in Fig. 5). A further point of difference between the more mature stage and the less mature stage is that the latter in cross-section shows dots in some of the polygons (Fig. 4)—a feature not so far seen in the more mature stage.

During the last stages of maturation to form the ripe sperm this well defined orientation can no longer be detected (Fig. 7). We cannot say from electron microscope studies whether this is because the chromatin becomes genuinely disorientated or because the longitudinal array becomes so closely packed that it cannot be detected—the nucleus is known to shrink a great deal in volume during maturation, its final diameter being only about 0.2 μ in the thread-like sperm. From birefringence studies one would certainly expect that the longitudinal orientation remains but becomes much more compact.

The structures described above appear equally clearly after fixation in osmium tetroxide or in formaldehyde. Limitations of space prevent the whole series being illustrated both from material fixed with osmium tetroxide and from material fixed with formaldehyde. Accordingly in the above series Figs. 1, 5 to 7 are from osmium tetroxide–fixed material and Figs 3 and 4 are from formaldehyde-fixed material, the latter section-stained with lanthanum nitrate (5). Fig. 2 shows one of many sections we have obtained of spermatid nuclei fixed in 45 per cent acetic acid; it demonstrates that even after this brutal, but classical, chromosome fixative the characteristic orientation of the chromatin is apparent.

In the course of our studies on the nucleus, we have observed that the part of the maturing sperm head surrounding the complex chromatin described above consists of a double sheath (Figs. 5 to 7). The two layers of this sheath, separated from each other by a membrane, each have a thickness of between 500 A and 1,000 A. The inner of the two layers appears to have a rather higher electron density than the outer one.

DISCUSSION

We cannot usefully discuss the fine structure of the sperm head chromatin in terms of the possible molecular structure of DNA until much more work has been done. We can, however, attempt to interpret the gross features observed—the parallel longitudinal lines in longitudinal section and the contiguous polygons in transverse section. It seems to us that these configurations are most likely to be produced by a bundle of slender and more or less straight tubes, which are orientated to run almost parallel to each other and to the long axis of the head. Analogous structures of this type (but on a much larger scale)

that come to mind are those of an elongated honeycomb and a plant vascular bundle. It must be emphasized that the resemblance to these structures is, of course, purely superficial. On the other hand the fine structure that has been demonstrated by Rudzinska and Porter in some specialised chromatin bodies in *Tokophrya* (13) shows a striking, although not complete, similarity to the fine structure we have described above in spermatid nuclei; this similarity is less superficial and it may even reflect an underlying similarity in the changes the chromatin is undergoing in the two cases.

We have attempted to trace in our micrographs the process by which the chromatin transforms itself from its apparently homogeneous state in the nuclei of secondary spermatocytes into the highly organised system of parallel tubes seen in fairly mature spermatid nuclei. It seems that as the nucleus begins to elongate the chromatin starts to form into sheets (Fig. 1). As maturation proceeds these sheets become more regular and extensive, and also more closely parallel to the long axis of the nucleus (Figs. 3, 5). Simultaneously with this, the sheets undergo a process of curling and infolding which results in the formation of the system of parallel tubes (Figs. 4, 6). However, we must be cautious about constructing a sequence of events from a series of static micrographs; the actual process may be quite different from that envisaged above.

Although there is little positive evidence we believe that the DNA in the spermatid nuclei must be contained in the walls of the tubes rather than in the central core of low density. The density of the core of the tubes is only slightly higher than that of the plain methacrylate in the section and since the DNA forms a considerable proportion of the dry mass at this stage it is difficult to see how it could be contained in cores of such low electron density. Moreover the density of the cores relative to the plain methacrylate is not appreciably increased by staining with lanthanum nitrate whereas the relative density of the tube walls is. Although lanthanum is not a specific nucleic acid stain, it is known to form an insoluble salt with DNA so that treatment with lanthanum nitrate would be expected to bring about an increase of density wherever DNA is present. It is necessary to say that we can only speak usefully of the location of the DNA in *fixed* spermatid nuclei; we have no information concerning its location in the *live* state.

In this study we have concentrated attention upon the nucleus of the spermatid and we have only incidentally made observations on the two sheaths of material surrounding the nucleus. In our preparations these sheaths have usually appeared rather poorly preserved and we do not wish to give a definite interpretation of them. The outer sheath is presumably cytoplasmic. As regards the inner sheath, it is worth noting that its granular and fibrous material does not increase in density during maturation to the extent that is characteristic of the complex tubular chromatin described above, and it seems most likely

that this sheath should be regarded as some ancillary body on the lines of the acrosome and the centriole. It is reminiscent of the paranuclear body described by Turian and Kellenberger (15) in gametes of *Allomyces*.

Grassé, Carasso, and Favard (7–9) have observed in developing sperm heads of a snail (*Helix pomatia*) chromatin configurations rather similar to those described above in locust sperm. They insist that the structures responsible are not "*rubans*" or "*membranes étroits*," but are instead filaments apparently homogeneous and of constant width (less than 100 A). Such a conclusion, though justified by their longitudinal sections, seems contradicted by some of their transverse sections (*e.g.* reference 8, Fig. 1, and reference 9, Plate X, Figs. 1 to 6) which are more compatible with the existence of folded sheets of the kind found in maturing locust sperm heads. In these figures there are very few of the dots which one would expect if the chromatin were all in the form of filaments. In their most recent paper (9) these filaments are said to form short meanders ("*boucles*"), then straighten, then form longer meanders, and finally straighten again during the course of sperm maturation. This may well be true, but on the other hand many of their micrographs could be more simply interpreted as showing the presence of long folded sheets of chromatin similar to those that we believe to exist in certain stages of locust spermatogenesis. The only electron micrograph in their papers which is completely contrary to this view is Fig. 7 of reference 8 (which is also Plate XII, Fig. 2 of reference 9) showing dots in a transverse section. If this appearance is found regularly in transverse sections of spermatid nuclei at a late stage of maturation, then these cannot at this stage contain sheets of the kind which we envisage in the locust. Unfortunately the interpretation of micrographs of snail spermatids is complicated by the fact that sperm development does not take place in well defined cysts (as it does in locust) and there is in consequence no good method of obtaining transverse and longitudinal sections which are known to represent the same stage of development. And for convincing reconstruction of the three-dimensional structure of the spermatid nucleus, one must have either serial sections or longitudinal and transverse sections of the same stage of development. It is, of course, to be expected that there will be some differences, possibly profound, between snail and locust in the fine structure of the chromatin during sperm maturation.

In conclusion it is interesting to note that most of the examples so far demonstrated in the electron microscope of fairly regular, complex, three-dimensional structure involving more or less all the chromatin have been among invertebrates, *e.g. Tokophrya* (13), a snail (9), a millipede (10), a locust (6). It remains to be seen whether similar structure occurs in vertebrate nuclei. In toad spermatids (1) there is certainly no sign of it (although the elongated sperm head might lead one to expect it); nor is there in the numerous electron micro-

graphs of mammalian spermatids prepared by various workers. On the other hand one would hardly expect a sharp distinction between vertebrates and invertebrates at this level of fine structure. Whatever may be the case regarding these extensive arrays involving virtually the entire chromatin, there seems to be no such distinction between vertebrates and invertebrates regarding the multi-strand chromosome core structures now being revealed by electron microscopy; for these have been seen in crayfish (11), pigeon, man, cat (3), and in locust. It also remains to be seen how all these relatively gross chromatin structures are related to the findings of x-ray crystallography on the underlying molecular structure of the desoxyribonucleoprotein forming the chromatin.

SUMMARY

1. In the heads of maturing sperm of a locust the chromatin becomes arranged in a highly regular manner so as to produce many parallel lines about 60 A thick in longitudinal section and contiguous polygons in transverse section.

2. This configuration appears after fixation in osmium tetroxide, formaldehyde, or acetic acid; intermediate stages in its development are illustrated.

3. These electron micrographs are interpreted to mean that, during sperm maturation, the chromatin becomes formed into sheets and then into tubes running parallel with the long axis.

4. In the mature sperm head we have been unable so far to detect this structure. This may be because the chromatin becomes so compact during the shrinkage of the nucleus which occurs during formation of the mature sperm.

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EXPLANATION OF PLATES

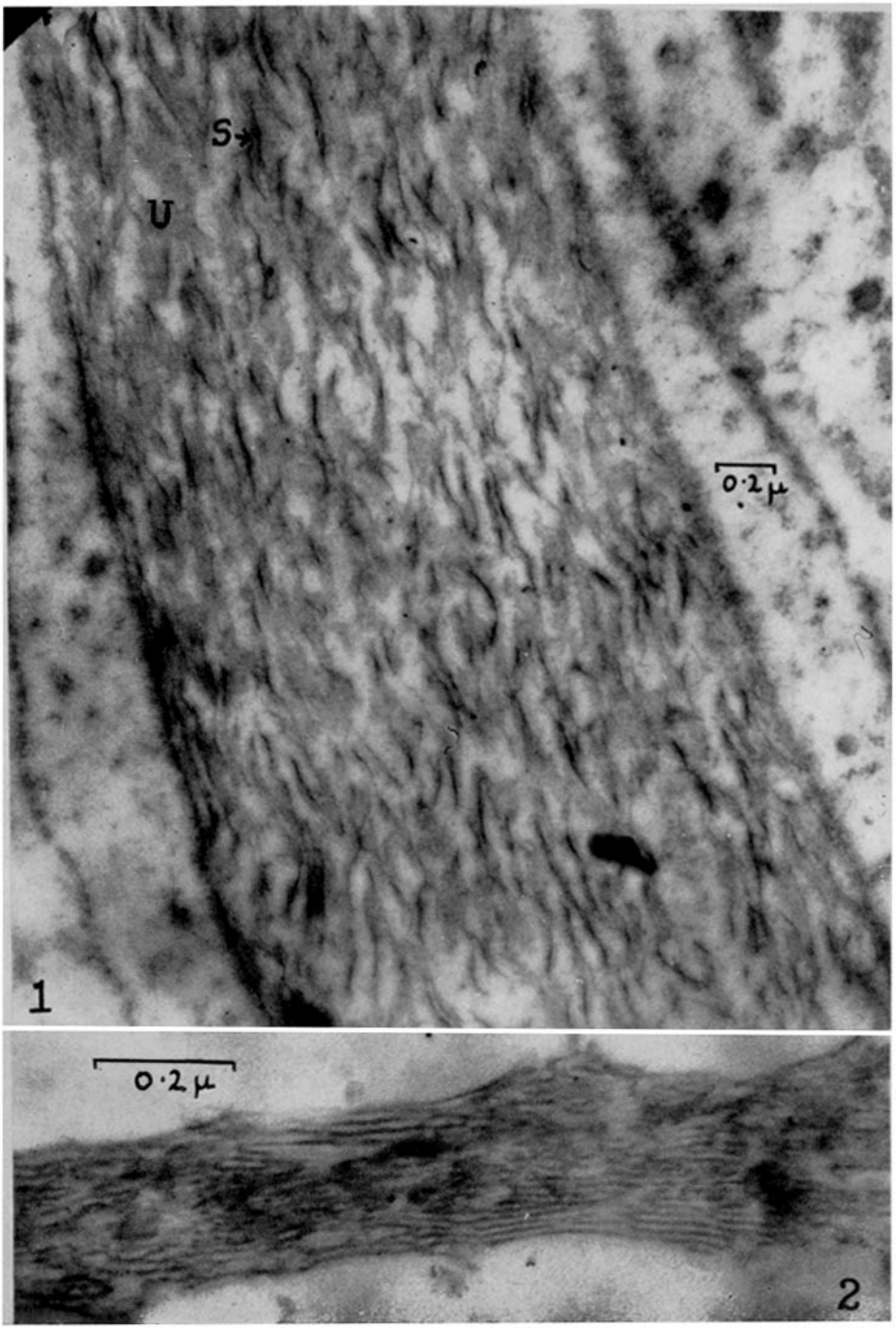
PLATE 23

FIG. 1. A longitudinal section of a locust spermatid nucleus, at an early stage of maturation, fixed in 1 per cent buffered osmium tetroxide.

The nucleus has already begun to elongate and this section shows it to contain a large number of dark lines up to 0.2 μ long. Most, but not all, of these lines run roughly parallel to the long axis of the spermatid. The thickness of the most electron-dense part of these lines varies from about 60 A to 100 A and there often occur irregular areas of less dense material on each side of the lines. These areas of only moderate density sometimes occur apparently unassociated with any dark line (*U*). The dark lines sometimes appear to split into two or more components (*S*). × 42,000.

FIG. 2. A longitudinal section of a spermatid nucleus at a late stage of maturation. This preparation was fixed in 45 per cent isotonic acetic acid and the section was stained with a saturated solution of phosphotungstic acid.

There are a large number of dark lines about 60 A thick running along the long axis of the nucleus. These lines are similar to those seen at this stage of maturity after osmic fixation (Fig. 5), although in this figure the general preservation is less good. The somewhat irregular outline of the spermatid shows that there has been some uneven shrinkage during preparation. × 95,000.



(Gibbons and Bradfield: Fine structure of nuclei during sperm maturation)

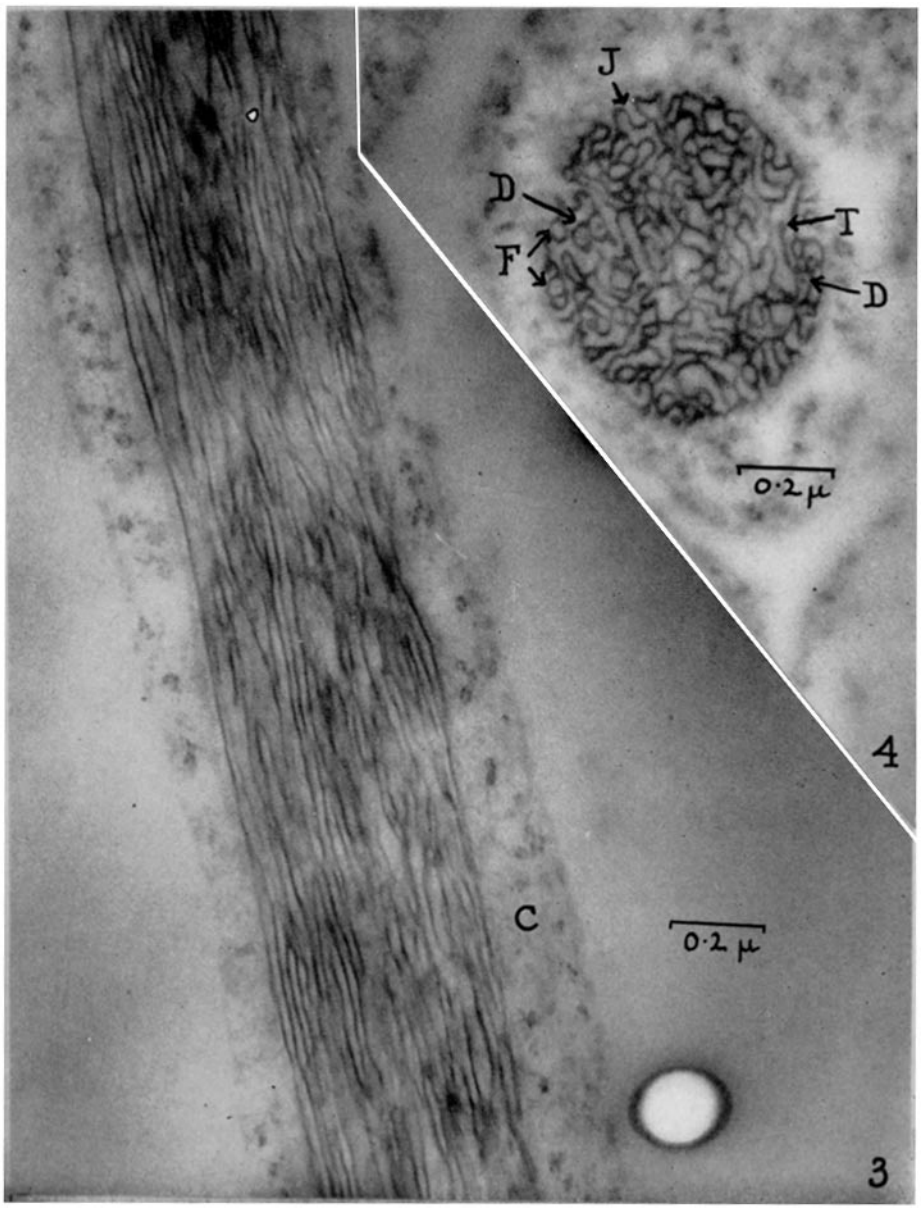
PLATE 24

FIGS. 3 and 4. These show longitudinal and transverse sections of spermatid nuclei from the same cyst of a follicle; this cyst was intermediate in maturity between that represented in Fig. 1 and that in Figs. 5 and 6. The preparation was fixed in 5 per cent buffered formaldehyde and the section stained in 2 per cent lanthanum nitrate.

FIG. 3. In this longitudinal section of a spermatid the electron-dense lines in the nucleus are longer, more regular, and more closely parallel to the long axis of the spermatid than those in the earlier stage of development represented in Fig. 1. The thickness of the lines is fairly constant at about 60 A and the maximum length contained within the section is about 1 μ. The lines sometimes appear to join but there is always the possibility of overlay. There is a sheath of cytoplasmic material (*C*) surrounding the nucleus; it does not appear well preserved by this fixation, and the two layers referred to in the text are not distinguishable. × 62,000.

FIG. 4. This transverse section of a spermatid shows a network of electron-dense lines within the nucleus. The network represents an intermediate stage in the formation of the mesh of contiguous polygons shown in the transverse section of a nucleus at a later stage of development (Fig. 6); it is made up of a large number of complete and incomplete polygons. Some of the smaller completed polygons have a regular finished appearance (*F*) while many of the larger polygons appear to be in the process of dividing themselves up into smaller units—sometimes this is done by an infolding of the sides (*J*) and sometimes by the outgrowth of an open loop which curls round on itself or links up with an opposite side. Some of these open loops appear to have a thickening at their free end (*T*). Note that some of the polygons have a conspicuous electron-dense dot in them (*D*); this feature has not so far been seen in later stages of maturation.

The thickness of the lines shown in this figure (about 80 A) appears rather greater than the thickness of the lines in the corresponding longitudinal section (Fig. 3). This is not a true difference however but an artefact caused by the micrograph shown in this figure being taken slightly under focus. × 62,000.



(Gibbons and Bradfield: Fine structure of nuclei during sperm maturation)

PLATE 25

FIGS. 5 and 6. These show longitudinal and transverse sections of spermatid nuclei from the same cyst of a follicle; this cyst was intermediate in maturity between those shown in Figs. 3 and 4 and that in Fig. 7. The preparation was fixed in 1 per cent buffered isotonic osmium tetroxide solution.

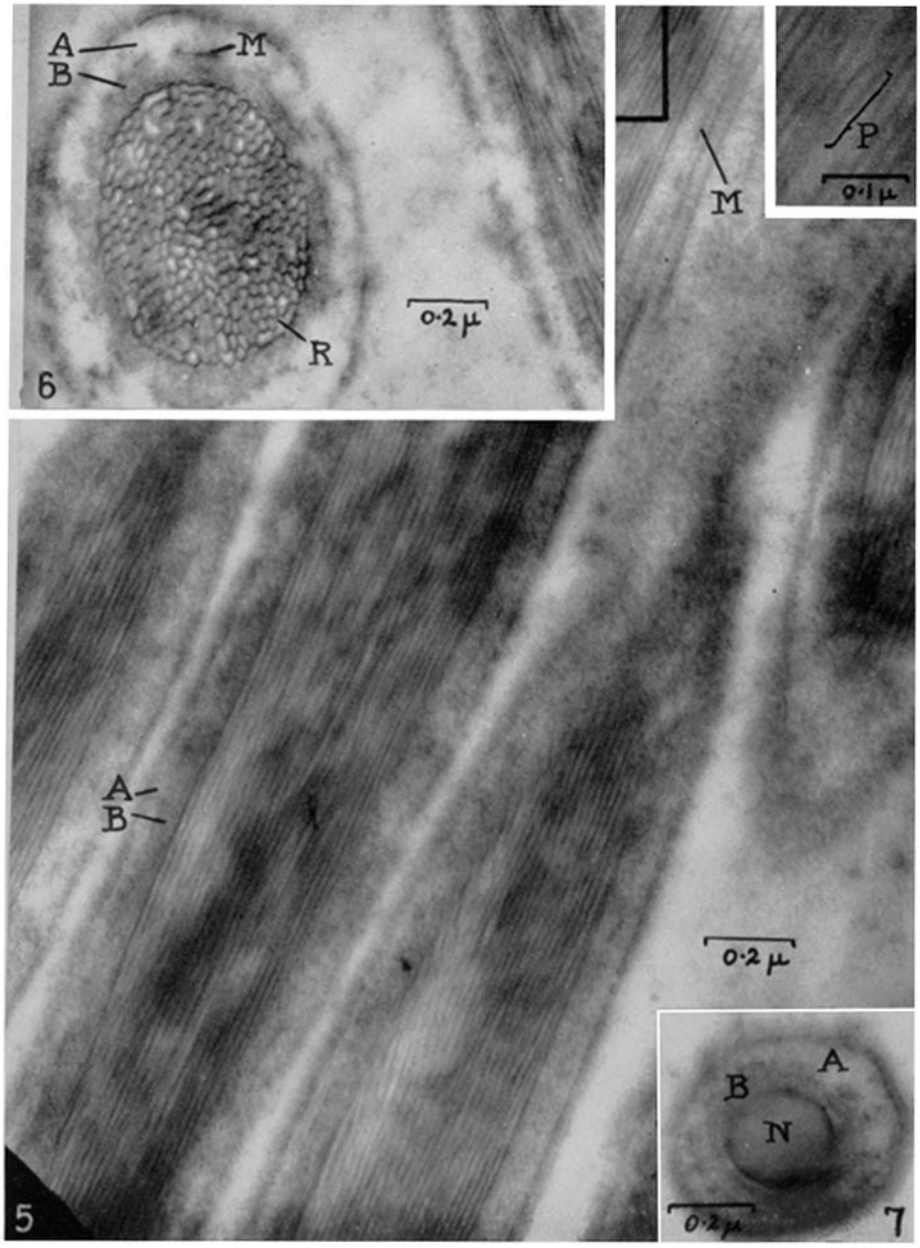
FIG. 5. This shows a group of spermatid nuclei cut in longitudinal section. In each nucleus there are a large number of electron-dense lines about 60 A thick. They are orientated to run almost parallel with each other and with the long axis of the spermatid. The lateral spacing between the lines varies from 180 A to 240 A (centre to centre). In some places there is evidence of a periodic variation in electron density along the lines (period about 75 A). The inset (top right) shows, at higher magnification, the area outlined, where there is such a variation in density along the line indicated (*P*). On the original print the variation, in this instance, can be seen for thirteen periods along the line. (Copies of the original print of this inset may be obtained from the authors on request.) The two layers (*A* and *B*) of the double sheath surrounding the nucleus do not show clearly in the micrograph because there is only a slight difference in density between them; in some places the membrane (*M*) between the layers can be seen. × 55,000 (inset × 110,000).

FIG. 6. This transverse section of a spermatid shows in the nucleus a large number (about 270) of contiguous polygons with electron-dense sides about 60 A thick and less dense interiors. The shape of many polygons is highly irregular and may well have been distorted at some stage of preparation. However, in that area of the nucleus in which the polygons appear most regular (*R*) they have the form of regular hexagons with a side length of about 180 A.

Note the double sheath of material surrounding the nucleus. The inner layer (*B*) shows a considerably higher density than the outer layer (*A*). The membrane (*M*) between the two layers is not well preserved and only fragments of it remain. × 55,000.

FIG. 7. A transverse section of the head of an osmium-fixed spermatid at a very late stage of maturation. The nucleus (*N*) has a uniform homogeneous appearance, and there is no apparent trace of the organised structure seen at earlier stages of maturation. Note the considerable shrinkage of the nucleus which has taken place since the stage of maturation represented by Fig. 6.

The double sheath (*A* and *B*) surrounding the nucleus is still present at this stage although the two layers appear to have almost the same density. × 55,000.



(Gibbons and Bradfield: Fine structure of nuclei during sperm maturation)