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HELICAL STRUCTURES IN THE CYTOPLASM OF THE MALE GERM CELLS OF THE DESERT LOCUST

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SUMMARY

A helical structure which had previously been reported in the cytoplasm of many cell types in the desert locust, *Schistocerca gregaria*, was found in large quantities in the developing male germ cells. The helices were isolated from these cells and negatively stained. The helices, which lie free in the cytoplasm of spermatogonia, primary spermatocytes and secondary spermatocytes (sometimes associated with a second type of helix, a double helix), closely invest the outer surface of the Nebenkern membrane in spermatids. As the Nebenkern elongates, the helices gradually disappear and are replaced by microtubules. The mitochondrial derivatives of the Nebenkern, which form part of the sperm tail, are in late spermatids completely surrounded by a sheath of parallel microtubules.

INTRODUCTION

In many cell types of the desert locust, *Schistocerca gregaria*, a helical structure of diameter of about 28 nm, pitch varying between 10 and 20 nm, and thickness 7·0 nm, has been reported.

Guthrie (1966) was the first to report the helices in nerve cells of *S. gregaria*, and he referred to them as 'neurotubules'. Odhiambo (1969), making the assumption that they were microtubules, wrote extensively about the helices, which he found in large numbers in the accessory reproductive gland of *S. gregaria*. In Odhiambo's micrographs, the helices are seen near the Golgi apparatus, and it was suggested that they arise from the Golgi and serve as a scaffolding which maintains the shape of the cells. A picture of the normal cylindrical microtubule coexisting with the helices was included, but Odhiambo made no comment about the apparent dimorphism of the microtubules.

Hawkes (1968) claimed that these structures were 'virus-like particles found in the cells of the desert locust', but did not provide evidence that the particles were infective, or that they contained RNA or DNA.

All 3 reports concern structures found in *S. gregaria* obtained from the Locust Research Centre, London, or the stock of animals kept in the Zoology Department, Cambridge, which were derived initially from the former stock. It is not known whether the helices are present in all strains of *S. gregaria*.

In electron-microscope studies, helical structures have, in the past, been reported

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from a variety of sources, such as the nuclei of *Amoeba proteus* and *Pelomyxa* (Pappas, 1956; Roth & Daniels, 1962), where they were later shown to contain RNA (Stevens & Prescott, 1966), the mitochondria of rat astrocytes (Mugnaini, 1964), a non-functional polymer of tobacco mosaic virus protein (Zaithin & Ferris, 1964), mixtures of saponin, lecithin and cholesterol (Glauert & Lucy, 1969), and 'vinblastine microtubules' (Marantz & Shelanski, 1970).

I decided to explore the relationship, if any, between the locust helices and the cylindrical microtubules since Guthrie (1966) and Odhiambo (1969) made the assumption that the two were identical. It would be of interest to establish whether the helices and microtubules are, in fact, isomeric forms. An explanation for the 2 forms might be that the helix represents a flexible or storage form of microtubule which could switch to the rigid, cylindrical form under appropriate conditions.

This paper will present information about the location and aspect of the helices in sectioned material (in particular in the maturing germ cells of the adult male locust), and their appearance when negatively stained.

METHODS

Sectioned material

Newly emerged males of the desert locust, *S. gregaria*, were used as a source of material. The locust were taken from the laboratory culture which has been maintained in the Zoology Department, Cambridge, for many years under a strict regime of a 12-h day (09.00–21.00 hours) at 35 °C, and a 12-h night at 25 °C. The insects are fed on young wheat plants, supplied fresh each day. They are very active during the day, and apparently in good health.

The testis was fixed, whole, in a 2·5 % solution of glutaraldehyde in 0·05 M sodium cacodylate buffer at pH 6·9, containing sucrose. Fixation in glutaraldehyde was for 1–2 h at room temperature. After glutaraldehyde fixation, the tissue was washed in several changes of buffer solution, and then postfixed for 1 h in 1 % osmium tetroxide solution also buffered with sodium cacodylate. The tissue was dehydrated in ethanol and embedded in Araldite.

A Huxley ultramicrotome and glass knives were used for sectioning. The sections were stained with saturated uranyl acetate in 50 % ethanol followed by lead citrate. A Philips EM 200 operated at 60 kV was used to examine the sections.

Negatively stained material

Three stains were used: (*a*) 2 % uranyl acetate in water, pH 4·8 (Barnicot, 1966), (*b*) 1 % phosphotungstic acid, in water, adjusted to pH 6·8 with NaOH, and (*c*) 2 % ammonium molybdate in water, adjusted to pH 6·8 with ammonium hydroxide (Glauert & Lucy, 1969).

One fresh testis follicle was burst in a drop of distilled water (about 0·2 ml) containing 0·002 M $CaCl_2$. The drop of water containing the cells was drawn up into a fine glass pipette twice or thrice to break the cells. A small drop of the suspension was placed on each grid (200-mesh grids coated with a film of carbon-stabilized Celloidin) and left for 1 min. The grid was washed gently with a few drops of distilled water, and dried with a small piece of filter paper. The grid was placed upon a drop of stain for 30 s and dried. It was then examined in the electron microscope.

OBSERVATIONS

Appearance of the helices in sectioned spermatogonia, spermatocytes and young spermatids

The helix appears to consist of a single coiled thread of thickness 7·0 nm. In some sections, this thread appears to be double (Fig. 1) but the double nature of the thread

in sectioned material may be a staining artifact. The diameter of the helix is 27–29 nm, and the pitch varies between 10 and 25 nm.

The core of the helices does not stain. Also noticeable is the unstained area of cytoplasm of about 20 nm surrounding each helix, and where helices are present in groups, the clear unstained area of the cytoplasm containing the helices extends for several microns.

In some spermatocyte cells, another type of helical conformation, which is smaller in diameter (20 nm) and appears to comprise 2 single threads 7·0 nm thick, is associated with the single helix (Figs. 1, 2). The double helix does not occur as frequently as the single helices. Double helices always lie in parallel array, whereas single helices may lie either as parallel bundles in hexagonal arrays or may be randomly scattered.

Negatively stained material

All stains gave essentially similar results, but clearest resolution was obtained with 2% uranyl acetate (Fig. 3). When stained in this way, the helix appears to consist of 2 parallel chains of subunits coiled upon a common axis. The subunits (measured directly from the photographic plates using a Nikon binocular microscope fitted with an eyepiece graticule) are approximately 3·0 nm in diameter, with subunits in the 2 parallel chains lying in register with one another. In a single chain there are about 19 subunits for each turn.

Some parts of the helix collapse when stained with uranyl acetate. In these collapsed regions, the 2 strands then appear twisted around one another. This does not occur when the helix is stained with sodium phosphotungstate or ammonium molybdate, where it is clear that there is only one order of coiling.

The double-stranded nature and the arrangement of subunits were clearly visible in every helix examined in preparations from more than 20 specimens of *S. gregaria*.

The 2 strands forming the helix can also be seen in preparations stained with sodium phosphotungstate or ammonium molybdate, but it is rare to see subunits in these preparations.

The distribution of the helices within the testis cells

The single helices occur in great abundance in the cytoplasm of the following cells: accessory reproductive gland cells (Odhiambo, 1969), and in spermatogonia, primary and secondary spermatocytes and early spermatids. The helices are present but less frequent in axons from the ventral nerve cord, the cells forming the wall of the testis follicle, oenocytes, and later spermatids. They are absent from mature spermatozoa (my own observations).

Rather than a relationship between Golgi apparatus and helices, as pointed out by Odhiambo (1969), I have noticed an association between mitochondria and helices, and, in spermatids in particular, this relationship is very striking.

In the early spermatids, mitochondria aggregate to form the Nebenkern, which eventually takes the form of 2 closely interdigitating, hemispherical mitochondria looking like a sectioned brain. During differentiation of the spermatids into spermatozoa, the 2 mitochondria forming the Nebenkern separate, become less convoluted, and

lengthen, ultimately forming the mitochondrial sheath of the spermtail (see Phillips, 1970, for review).

At the early spermatid stage, the Nebenkern is invested with helices, which lie close to the outer Nebenkern membrane (Fig. 6). They are spaced at a distance of approximately 90 nm from one another, and lie parallel like lines of latitude around the Nebenkern.

The helices persist in their association with the Nebenkern while the flagellum of the early spermatid forms, and during separation and the initial stages of elongation of the Nebenkern (Figs. 7, 8). As the Nebenkern lengthens further in the maturing spermatids, the helices gradually disappear and are replaced by microtubules lying close to the 2 mitochondria. The microtubules increase in number as the cells mature and elongate. Finally the mitochondria, now devoid of internal cristae, become surrounded by a sheath of microtubules which run parallel to the direction of elongation along the developing flagellum.

During the maturation of the spermatids, the nucleus undergoes a similar process of elongation to the Nebenkern, but is seen associated only with microtubules, and never with helices. Nuclear elongation begins after the Nebenkern has elongated appreciably, at a stage when the chromatin within the nucleus is beginning to condense.

The helices are sometimes seen in close association with single microtubules. In Fig. 4 a helix is seen in parallel with a spindle microtubule, and Fig. 5 illustrates an apparent continuity between the 2 structures.

DISCUSSION

The negatively stained preparations demonstrate that the helices do exist in unfixed material, and are not just an aggregate formed during glutaraldehyde or osmium fixation. The double nature of the helix revealed by negative staining provides a clue to the relationship of the double and single helices seen in sectioned material. The double helix may be another configuration for the double chains of subunits comprising the single helix, but wound contralaterally rather than in parallel.

The circumstantial evidence that the helices are related to microtubules is quite compelling. Their numbers seem to correspond, not with the number of microtubules present in the cells, but rather with the amount of microtubular construction and destruction in cells. Where cells are undergoing division or changes of shape, as in the spermatogonia and spermatocytes, and early spermatids, helices are very numerous. Perhaps the helix occurs as an alternative, flexible form of microtubules in cells where a rapid turnover and readily available pool of microtubules are required, to build and destroy scaffolding quickly.

The curved microtubule in Fig. 4 may represent an intermediate state between open helix and microtubule. In particular, the intimate association between the Nebenkern and the helix suggests that the helix is a 'pro-microtubule' organizing itself into the correct position prior to the final elongation of the Nebenkern along the spermatid tail. Kessel (1966, 1967) describes a basket of microtubules surrounding the nucleus of the early spermatid in dragonfly and grasshopper, prior to nuclear elongation. After

elongation of the nucleus in these insects, microtubules persist around the mitochondria which constitute part of the sperm tail, as I have described in the locust *Schistocerca.*

A recent paper by Fawcett, Anderson & Phillips (1971) points out the ubiquity of microtubules around the condensing nucleus and elongating Nebenkern of elongating spermatids in several species, but their observations do not suggest that the microtubules themselves are responsible for the elongation or shape determination.

None of these authors describes the very early stages of microtubule association with the elongating organelles, but it is difficult to believe that they could have overlooked such a conspicuous structure as the helices, if such were present. In this case, *S. gregaria* must have a type of microtubule peculiar to itself.

The helix is larger in diameter than the cyclindrical microtubule. If the two are alternative forms, one must suppose that some switch in environmental conditions within the cells effects the conversion of one form into the other. Thirteen linear protofilaments constitute the wall of a cylindrical microtubule (Ledbetter & Porter, 1963; Pease, 1964; Gall, 1966). Each filament is made of subunits, which are also arranged in a shallow helix around the tubule (Porter, 1966). There is also evidence for lateral as well as longitudinal linkage between subunits in the case of lung fluke cytoplasmic microtubules (Burton, 1970). The helix may in fact be a coil of 2 parallel chains of subunits (Burton, 1970). There are about 19 subunits per turn in the locust single helix. A change from helix to microtubule would require a diminution of the diameter of the helix, and extensive cross-linkage between adjacent gyres, as Burton (1968) suggested. Burton (1966, 1970) proposed a change from open spring to firm cylindrical structure without a change in the number of subunits per turn, which would result in a microtubule of slightly greater diameter than the open spring. This relationship in the locust clearly does not exist, for the helices are of greater diameter than the microtubules. However, Bensch & Malawista (1969) observing microtubules of greater diameter than the ordinary type (28 compared with 25 nm) in cells treated with vinblastine, suggest that, 'the difference in dimensions may be caused by rearrangements of the binding sites in the tubule protein'. Tilney & Porter (1967) suggested structures for 2 stable forms of microtubules of different diameters which they observed after degrading the microtubules in Heliozoa; this suggests that the same protein could be capable of coiling in various ways to make structures of different dimensions, as would be required in the *Schistocerca* cells.

I commented above upon the clear unstained area surrounding the helices, which excludes small particles such as ribosomes, and larger organelles such as mitochondria. The exclusion of other organelles suggests that the helices are embedded in a matrix which is not revealed by standard fixation and staining techniques for electron microscopy. The microtubules are surrounded by clear spaces, narrower in extent than the space around the helices. The existence of material in the perimicrotubular spaces can be revealed by staining with lanthanum (Lane & Treherne, 1970). Clearly, the nature of such a matrix surrounding – and always associated with – single helices, double helices and microtubules, must affect profoundly the conformation of the structure which we see in the sectioned cells. It is not unlikely that this matrix governs the change

between one form of assembly of the protein to another – a change of the type suggested by Tilney & Porter (1967).

Further discussion about the conversion of helices into microtubules is not profitable at this stage, for there is only circumstantial evidence that there is any relationship at all between the two structures. To establish whether in fact such a relationship exists or not, experiments using colchicine and vinblastine have been carried out and will be reported later.

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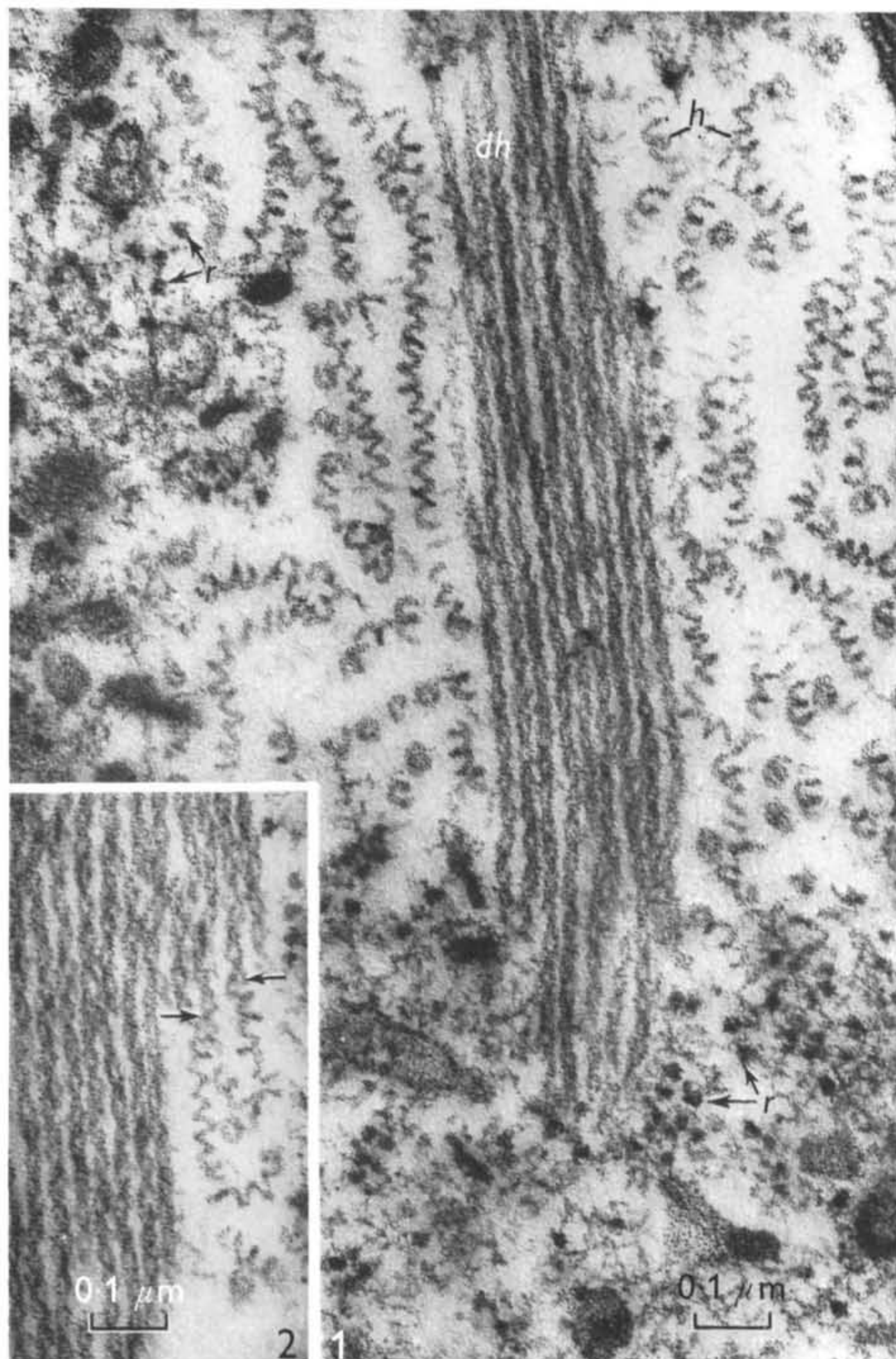

Fig. 1. Single (*h*) and double helices (*dh*) are seen together in the cytoplasm at the periphery of a primary spermatocyte. The clear area of cytoplasm, containing helices only, extends for about 1·5 μm in each direction, and is bordered by cytoplasm containing ribosomes (*r*) and other organelles. × 100000.

Fig. 2. Single and double helices in the cytoplasm at the periphery of a secondary spermatocyte. The single helix is seen to be continuous with the double helix (arrows). × 100000.

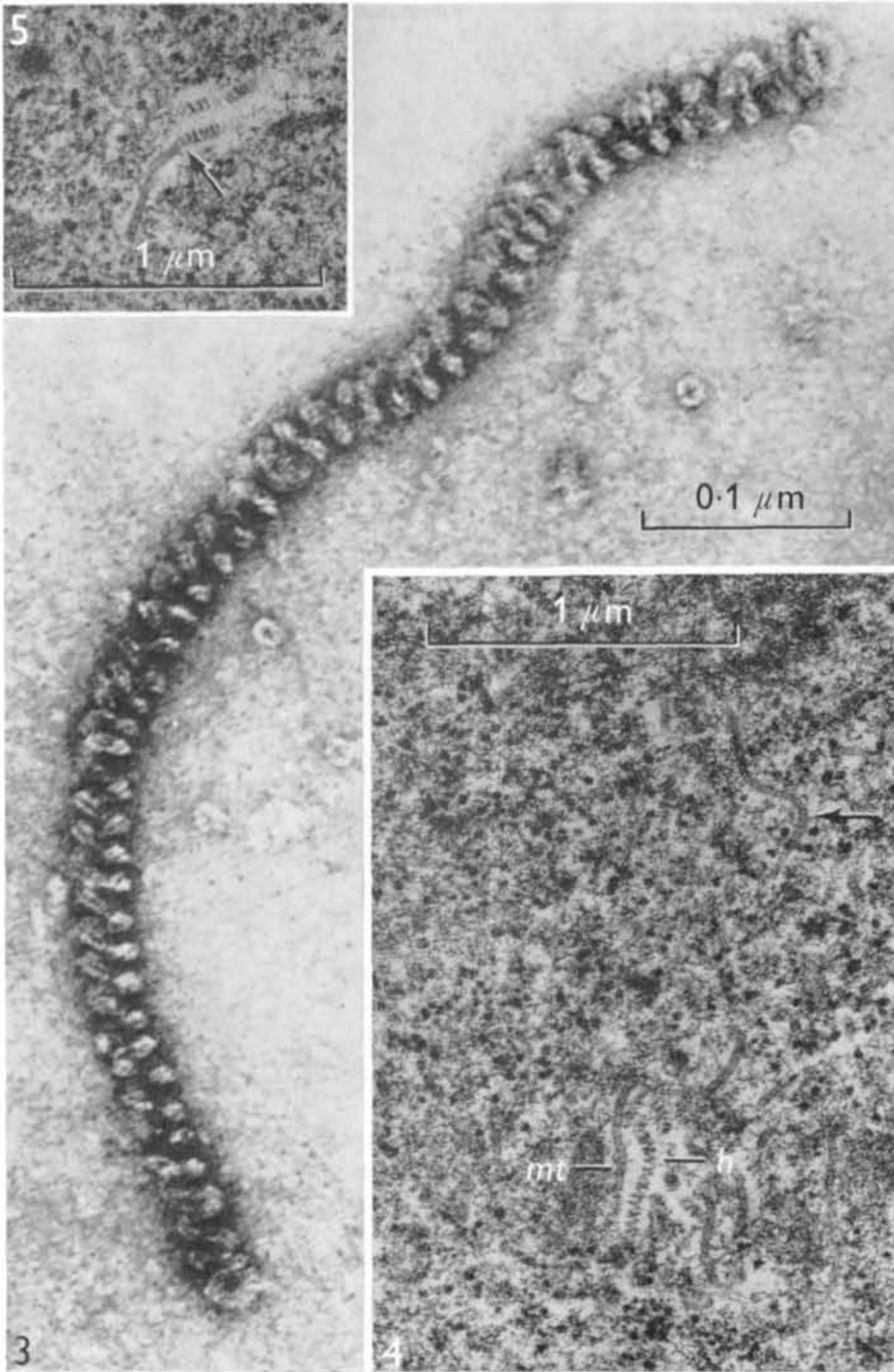

Fig. 3. Single helix negatively stained with uranyl acetate. The helix, which has collapsed upon itself during staining, is clearly seen as a structure made of 2 parallel, coiled chains of subunits. The individual subunits are visible in parts of the chain. × 300 000.

Fig. 4. A single helix (*h*) is seen lying parallel to a cylindrical microtubule (*mt*) in the second meiotic spindle. The long axis of the spindle runs from top to bottom of the micrograph. The very curved microtubule (arrow) may represent a stage intermediate between helix and microtubule. × 40 000.

Fig. 5. A single helix is apparently continuous with a microtubule in the second meiotic spindle (arrow). A chromosome can be seen at top left of the micrograph. × 40 000.

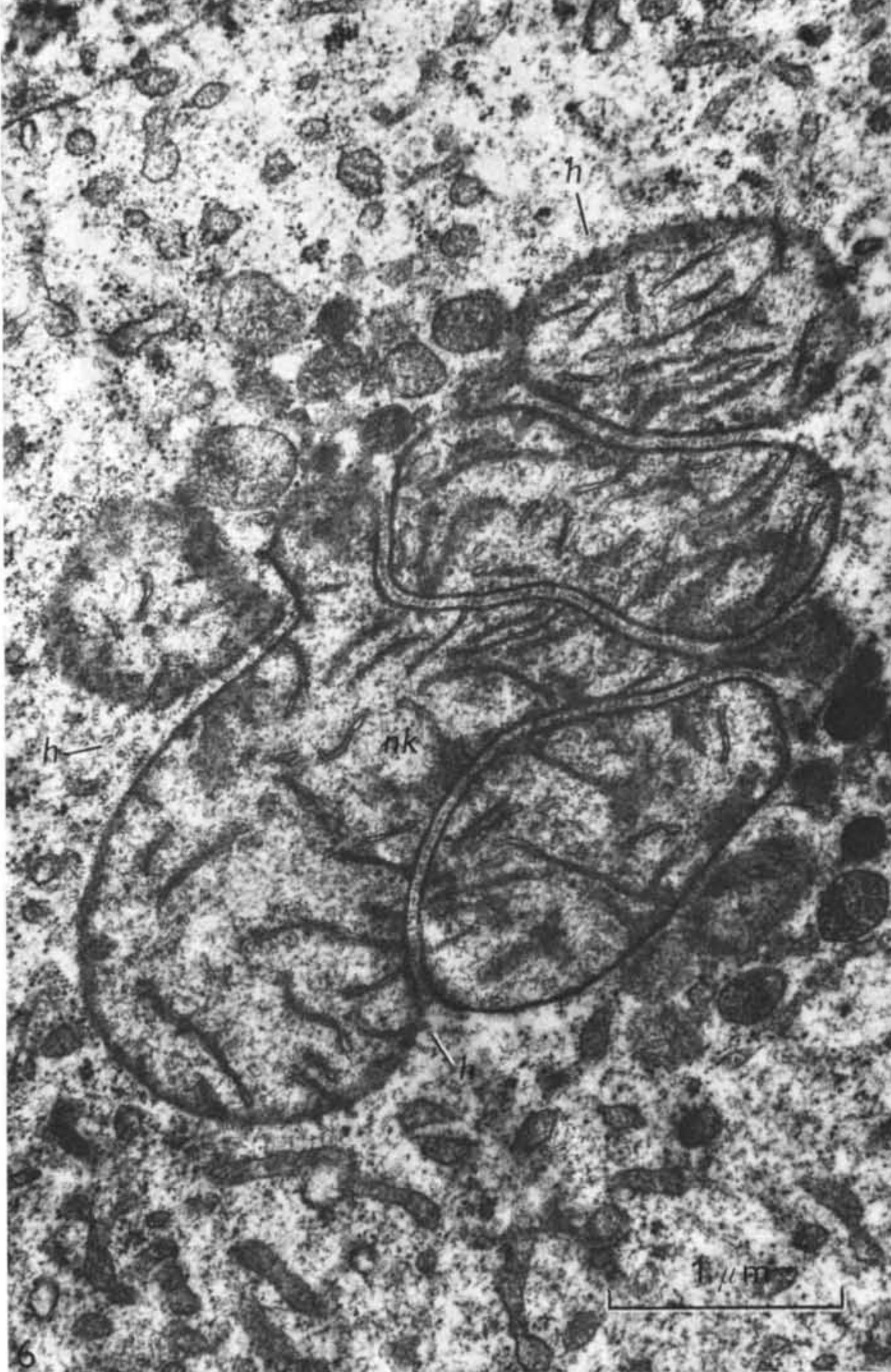

Fig. 6. The mitochondria in a young spermatid are aggregating to form a Nebenkern (*nk*). Helices (*h*) can be seen investing the surface of these mitochondria, but are not present elsewhere in the cell. × 28 000.

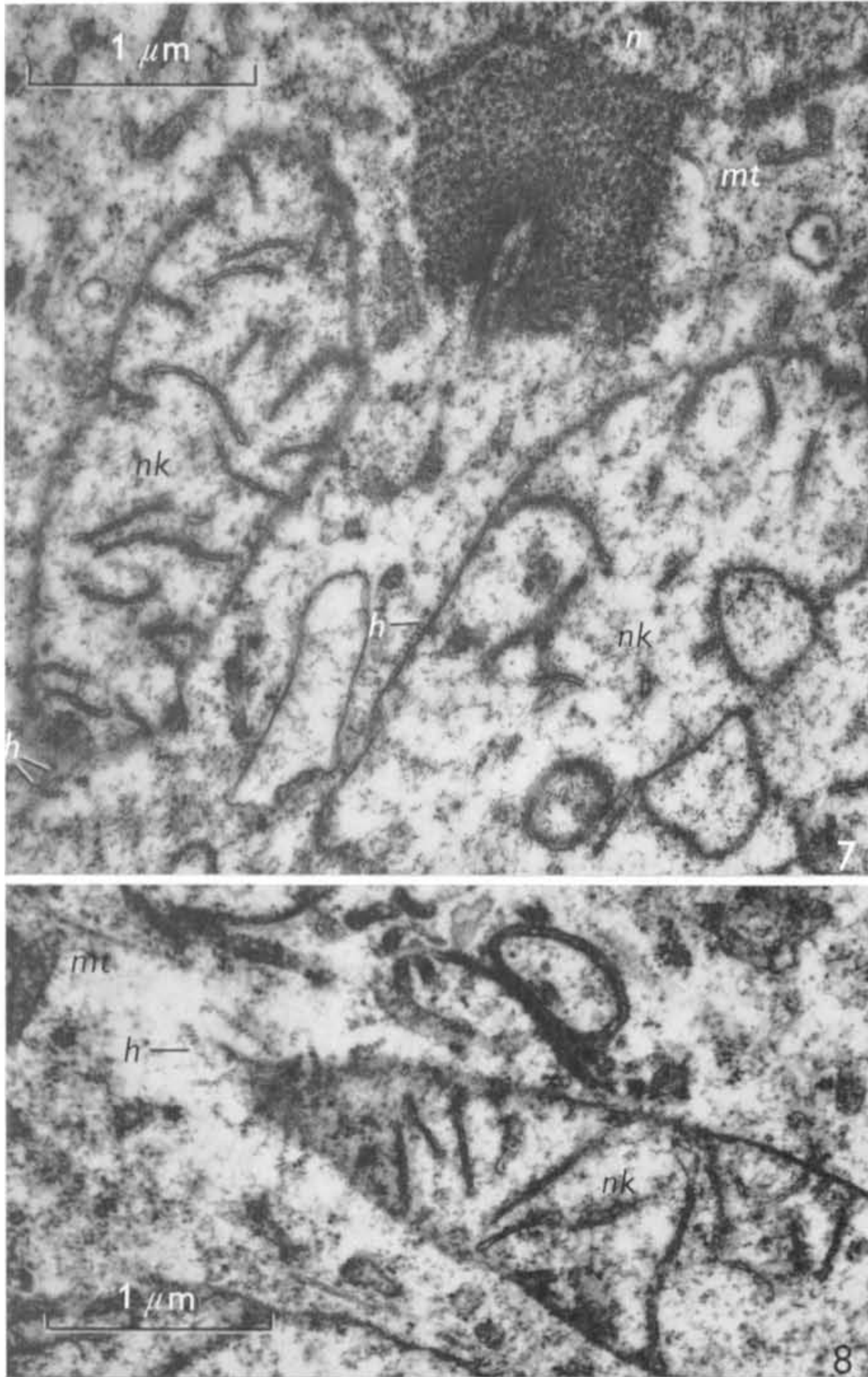

Fig. 7. The flagellum (which is sectioned obliquely) is now lying centrally between the 2 mitochondria forming the Nebenkern (*nk*). The mitochondria have separated slightly. The helices (*h*) are seen associated with the Nebenkern surface as before. A microtubule (*mt*) is adjacent to the pericentriolar material, and the nucleus (*n*) of the spermatid is visible at right. × 28 000.

Fig. 8. The mitochondria (*nk*) are now considerably elongated. The flagellum, sectioned obliquely, can be seen lying between them. Microtubules (*mt*) now run parallel to the direction of elongation, while helices (*h*) are still closely associated with the Nebenkern, at the end distal from the nucleus (not visible). × 28 000.