The Spermatid and the Sperm of the Crab, Paratelphusa spinigera.

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With Plate 32.

INTRODUCTION.

The Decapod spermatogenesis has attracted during the past a large number of workers, some of whom have given excellent descriptions of nuclear detail and centrosome, &c. To the best of my knowledge none has given an account of the Golgi apparatus and its destiny in the process of sperm-formation. A few have described mitochondria, but it is not possible to accept most of these descriptions on account of the unsuitable technique employed.

With regard to spermatogenesis generally, two of the most important contributions made during the last few years are (1) that the mitochondria (or their product) always form an envelope at least of some part of the axial filament, and (2) that the Golgi apparatus is concerned in the formation of the acrosome. But on a perusal of the literature on Decapod spermatogenesis one finds that there is no mention of the acrosome, or indeed of the Golgi apparatus; and in a few cases in which the mitochondria have been described they are not always said to lie in the tail region. Indeed the existing accounts are not only absolutely insufficient in this respect, but all writers actually describe certain bizarre structures which are unheard of in typical spermatogenesis. Consequently the Decapod sperm has been hitherto regarded as a weird cell, very different from the typical spermatozoon.

In view of the above I undertook some years ago the study of some of these so-called atypical spermatozoa, and in this first paper of the series an account of the spermatogenesis of a crab
is offered with particular reference to the transformation of the spermatid into the sperm. No attention has been paid to nuclear changes, for which reference may be made to Fasten's excellent paper (1918).

I have to thank Mr. Sukh Dayal Malik, M.Sc., for doing in ink all the diagrams published in this paper.

**Previous Work.**

Fasten (1914) has reviewed the whole literature on the Decapod spermatogenesis. A reference to this paper makes it clear that practically all the workers have confined themselves to the Macrura and the Anomura. Virtually nothing had been done with the Brachyura till Fasten (1918) himself published a paper on the spermatogenesis of the Pacific Coast edible crab, Cancer magister. For a proper understanding of the somewhat complicated process of spermatoeleosis in Paratelphusa, it is essential to summarize below Fasten's account of the same process in Cancer.

The spermatids produced are, at first, small and their nuclei contain large masses of chromatin. The cytoplasm is homogeneous throughout and within it a rather prominent centrosome is found. Gradually these chromatin masses disappear till ultimately only one is left. This may be said to be a nucleolus-like body which resembles a karyosome.

At about this time a densely staining mass makes its appearance in the cytoplasm. This mass has been called a mitochondrial mass by Koltzoff (1906) and Binford (1913). It stains like the chromatin of the nucleus. Fasten does not consider this mass mitochondrial in nature, especially because 'in the cells under consideration no traces of mitochondria have been observed in the earlier stages of the maturation'. On the other hand, he considers it likely that it consists of chromatin which has diffused out of the nucleus.

The nucleus wanders to one pole of the spermatid, while at the opposite pole a clear vacuole makes its appearance. Sometimes two clear vacuoles may be seen, but these later flow together into a single one. At the same time the mitochondrial mass wanders in between the nucleus and the vacuole, and
ultimately fills this entire space. The centrosome increases somewhat in size and takes a position in the centre of the mitochondrial mass.

The mitochondrial mass now transforms into a ring, and the centrosome comes to occupy the centre of its inner open space. The upper portion of the nucleus also becomes located in this space. At the same time the karyosome-like body migrates upward to the middle of the upper portion of the nucleus until it comes to lie directly below the centrosome.

Simultaneously with the last-mentioned changes, a second vacuole makes its appearance in the anterior extremity of the original first, or primary vacuole. During this time the centrosome and the karyosome-like body of the nucleus unite, and elongate into a rod-like structure, the so-called central body which penetrates the inner or proximal portion of the second vacuole.

Now an opening makes its appearance in the middle of the outer or distal end of the second vesicle (vacuole). Simultaneously with this, the central body elongates still more and its outer extremity seems to hollow out into a thin tube which soon connects up with the distal opening in the secondary vesicle. As the outer end of the central body hollows out, a ring of densely staining material, the chromatin ring, makes its appearance around the outer opening of the second vesicle.

Going hand in hand with these modifications are those which take place in the mitochondria-like ring and the nucleus. These two elements fuse into a single nuclear-mitochondrial cup. At the same time the second vesicle fits more compactly into the first vesicle. Now the radial arms or rays of the spermatozoon make their appearance. They originate as outgrowths from the nuclear-mitochondrial cup, and in the finished state they are stout structures with pointed extremities. In the mature spermatozoa the rays are tightly coiled around the nuclear-mitochondrial cup.

Fasten has also described in the cytoplasm of the primary spermatocytes two densely staining chromatoid bodies, each surrounded by a clear area. During the first meiotic division the chromatoid bodies pass undivided to opposite poles of the
cell, so that each secondary spermatocyte contains a chromatoid body. During the second meiotic division it passes undivided to one pole, resulting in the formation of two classes of spermatids, one of which contains the chromatoid body, while the other is without it. The chromatoid body is soon expelled from the spermatids which contain it, thus making all the spermatids alike in structure and appearance.

Fasten worked almost exclusively with smears. Sectioned material was also used, but the smears were of the greatest service and virtually all the deductions and illustrations were made from them. Smears were fixed with Bouin's fluid while the material to be sectioned was fixed with Flemming's strong and the Meves-Duesberg modified Flemming, all of which, it may be noted, contain acetic acid.

From the above review it becomes clear that in practically the only paper available on the spermatogenesis of the crab no mention is made of the acrosome and the Golgi apparatus. The mitochondria-like mass is considered to be chromatinic in origin, and two unusual structures, the vesicles and the chromatin-ring, have been described. These are the problems which are investigated in the present paper.

**Material and Methods.**

Specimens of the crab, *Paratelphusa spinigera*, were obtained a number of times from a fresh-water stream in Kapurthala (Punjab). On their arrival they were dissected immediately. Sectioned material proved unsatisfactory for the study of spermatids. In these cells the various cell-components are so arranged that one section fails to show them all properly. Smears had, therefore, to be prepared, and they proved admirable for the study of spermatogenesis. The smears on slides were fixed from one to two hours in jars containing Champy's fluid, Flemming-without-acetic, and the same diluted with an equal amount of water. After washing them in running water for some time they were mordanted and stained with 0.5 per cent. haematoxylin in the usual way. Benda's alizarin and crystal violet were also used after keeping the Champy-fixed smears first in a mixture of one part pyroligneous acid and two parts
SPERM OF PARATELPHUSA

of 1 per cent. chromic acid, and then in 3 per cent. potassium bichromate and iron-alum for varying periods. Both these methods yielded very satisfactory preparations. Bouin's fluid was used for control. Fresh sperms from the vas deferens were also studied.

Observations.

In the earliest spermatid the nucleus is perfectly spherical and shows a faintly staining medullary region and a darker cortical one (figs. 1–8, Pl. 32). Sometimes a small, darkly staining granule, corresponding to the nucleolus or the karyosome of Fasten, is found embedded in the nuclear reticulum (fig. 4, Pl. 32). In the cytoplasm lie the centrosome, the mitochondria, and the Golgi elements. The first is a darkly staining granule, and its position in the cell is by no means constant. The mitochondria are extremely delicate vesicles scattered throughout the cytoplasm (figs. 1–8, Pl. 32). They stain very lightly with haematoxylin or with crystal violet. Each mitochondrion shows a slightly chromophilic periphery and an absolutely chromophobic interior. They can be very aptly compared to small soap bubbles. There are a few Golgi elements lying close together usually near the nucleus. These stain much more densely than the mitochondria. Each appears in the form of a ring with a darkly staining periphery and a lightly staining interior (fig. 1, Pl. 32). Sometimes a Golgi element may appear in the form of a crescent (fig. 8, Pl. 32), or a horseshoe (fig. 7, Pl. 32), but these forms are very rare.

From now onward four changes begin to take place simultaneously in the spermatid. The first concerns the nucleus and the other three occur in the centrosome, the mitochondria, and the Golgi elements. The thin, peripheral, darkly staining area of the nucleus becomes prominent. It gradually expands inwards till the central, lightly staining area is considerably reduced (fig. 9, Pl. 32). The latter ultimately disappears altogether and the whole of the nucleus stains darkly and uniformly (figs. 11, 12, 13, 14, and 16, Pl. 32). The centrosome first becomes rod-shaped (figs. 2 and 4, Pl. 32) and then divides into two (figs. 3, 5, 6, and 8, Pl. 32). The mitochondria grow and become more resistant to acetic acid. Nevertheless, they
continue to stain but lightly. In many cases the cytoplasm is so densely packed with them that it presents the appearance of a honeycomb (figs. 1 and 5, PI. 32). Very often very large mitochondria appear which undoubtedly are formed by the running together of smaller ones (fig. 6, PI. 32). The Golgi rings come to be arranged more closely together and begin to stain more densely. Consequently they form one oval, or spherical, or kidney-shaped, and densely staining compact body in which, however, the individual rings may still be observed for a long time (figs. 2–8, PI. 32). This body is destined to form the acrosome and corresponds with the 'mitochondria-like mass' of Fasten.

The process of the running together of the mitochondria continues till a large, clear vesicle is formed (figs. 9 to 13, PI. 32) corresponding to the first or primary vesicle (or vacuole) of Fasten. A few mitochondria are left over and do not share in the formation of the mitochondrial vesicle when it first appears. There is no evidence, however, that these mitochondria are sloughed off. On the other hand, it appears that they too are gradually absorbed into the big vesicle.

Simultaneously with the completion of the mitochondrial vesicle the two centrosomes place themselves at or near its base (figs. 12 and 13, PI. 32). Soon after one of these establishes itself on what will become the distal or posterior border of the vesicle (fig. 14, PI. 32). It soon becomes ring-like—the so-called 'Chromatin-ring' of Fasten (figs. 15 to 18, PI. 32). From now onward this will be called the distal centrosome. Simultaneously with this change the hitherto spherical nucleus becomes flattened out in many cases (figs. 14, 16, 17, and 22, PI. 32).

Soon after this the nucleus becomes cup-shaped (figs. 19 and 20, PI. 32). The nucleolus is first reduced to a faint streak (fig. 24, PI. 32) and then disappears. The mitochondrial vesicle fits into the cavity of the cup, on the surface of which lies the acrosome (figs. 19 to 21, PI. 32). The proximal centrosome, which was hitherto a granule, grows into a small vesicle with a thick periphery and a hollow interior. It places itself at the bottom of the mitochondrial vesicle and undoubtedly corresponds to the secondary vesicle of Fasten.
A very interesting change now comes over the acrosome. It first expands into a band (figs. 21 and 22, Pl. 32) which is rapidly converted into a ring (figs. 23, 24, and 25, Pl. 32). Even at this late stage an indication of the origin of the acrosome from the Golgi rings may be furnished by the presence of vacuoles inside it (fig. 28, Pl. 32). Gradually the acrosomal ring becomes less prominent as it begins to fuse with the margin of the nuclear cup, till in the ripe spermatozoa the two structures cannot be distinguished from each other (figs. 26, 27, and 28, Pl. 32). Thus is formed the nuclear-acrosomal cup corresponding to the nuclear-mitochondrial cup of Fasten.

Simultaneously with the above changes the nuclear cup becomes deeper and the mitochondrial vesicle fits more closely into its cavity. Consequently the margins of the cup touch the periphery of the ring-like distal centrosome, which now forms a very efficient plug keeping the vesicle well pressed into the cup (figs. 26 and 27, Pl. 32). At the same time the axial filament, corresponding to the central body of Fasten, grows from the bottom of the proximal centrosome, and, after piercing it and the mitochondrial vesicle, stops just below the distal centrosome. At its distal end the axial filament shows a very small darkly staining, transverse piece. This would, it appears, fit into the middle of the ring-like centrosome, thus supplementing the function of the latter of keeping the mitochondrial vesicle well pressed into the nuclear-acrosomal cup.

The process of spermateleosis is now completed. The spermatozoon, when looked at from the bottom (fig. 29, Pl. 32), appears as a disk. The margin of the disk stains deeply and represents the fused nucleus and the acrosome. Within this is the very faintly staining mitochondrial vesicle, in the centre of which lies the vesicular proximal centrosome, containing a darkly staining granule, the axial filament.

It has been mentioned above that the ring-like acrosome fuses with the margin of the nuclear cup so that in the ripe sperm the two structures cannot as a rule be distinguished from each other. But when fresh sperms from the vas deferens are studied in a drop of neutral-red solution two thickenings may be very often observed lying just within the margin of the nuclear cup.
These undoubtedly represent the attenuated acrosomal ring which at the point of curvature becomes visible (fig. 80, Pl. 32). Similarly in fixed preparations of the testis (e.g. Kolatchev) the two thickenings have been very commonly observed. All the other structures, namely, the nucleus, the axial filament, the proximal and the distal centrosomes, and the mitochondrial vesicle can be likewise observed in fresh sperms studied in a drop of water or normal saline.

Fasten has described radial arms or rays in the spermatozoon of Cancer. 'They originate as outgrowths from the nuclear-mitochondrial cup, and in the finished state they are stout structures with pointed extremities.' In the sperm of the crab, Paratelphusa spinigera, I have never seen rays so big as figured by Fasten. But quite often, just below the margin of the nuclear-acrosomal cup, short but prominent processes are given out which probably correspond to the rays of Fasten (fig. 26, Pl. 32). Sometimes a third process may be seen at the proximal end of the sperm (fig. 28, Pl. 32).

Lastly, two points of minor importance may be mentioned. Firstly, in some cases, the mitochondrial vesicle may contract so that an empty space appears between it and the nuclear cup (fig. 25, Pl. 32). Secondly, the proximal centrosome may become elongated (figs. 25 and 27, Pl. 32) and may even extend from the bottom of the mitochondrial vesicle right up to the distal centrosome (fig. 26, Pl. 32).

Discussion.

I. Spermateleosis.

To sum up: The spermatozoon of the crab under discussion is a deep cup. The wall of the cup represents the nucleus with the ring-like acrosome fused with its margin. The mouth of the nuclear cup is very efficiently plugged by the ring-like posterior or distal centrosome. This serves to keep the mitochondrial vesicle well pressed into the nuclear cup. At the bottom of the cup in the lower region of the mitochondrial vesicle lies the vesicular proximal or anterior centrosome. From the bottom of this arises the axial filament which, piercing it and the mitochondrial vesicle, stops just below the distal centrosome.
A careful comparison of this description with that given by Fasten for Cancer will reveal the very interesting fact that the two tally in every detail. But, since he was using acetic acid and since the fate of the Golgi apparatus and the mitochondria in typical spermatogenesis was not yet fully understood, he failed to homologize the various sperm-components properly. This can be brought out very clearly by the study of the following table:

<table>
<thead>
<tr>
<th>Cancer (Fasten)</th>
<th>Paratelphusa (Nath)</th>
</tr>
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<tbody>
<tr>
<td>2. Primary vacuole or vesicle.</td>
<td>2. Mitochondrial vesicle.</td>
</tr>
<tr>
<td>3. Chromatin ring.</td>
<td>3. Distal or posterior centrosome.</td>
</tr>
<tr>
<td>4. Secondary vacuole or vesicle.</td>
<td>4. Proximal or anterior centrosome.</td>
</tr>
<tr>
<td>5. Central body.</td>
<td>5. Axial filament.</td>
</tr>
</tbody>
</table>

Fasten never saw the mitochondria till they had run together to form a vesicle with contents sufficiently thick to resist the action of acetic acid. Not knowing how it was formed he called it the primary vacuole or vesicle. It need hardly be pointed out that the progressive increase in resistance of the mitochondria to acetic acid during spermateleosis is now a well-established fact (see Nath, 1926, for references). It will be recalled that in the spermatid of Paratelphusa the Golgi elements have been described as rings, or very rarely as crescents or horseshoe-shaped structures closely aggregated together. These soon give rise to a compact, deeply staining body destined to give rise to the acrosome. In spite of the acetic acid used by Fasten this body was not washed out in his preparations. He saw it and so did Koltzoff (1906) and Binford (1913). They considered it to be a mitochondrial body, although Fasten thought that it was chromatinic in origin. Similarly Fasten misinterpreted the proximal and the distal centrosome as the secondary vesicle and the chromatin-ring respectively. The central body was considered by Fasten as a structure formed by the union of the centrosome and the karyosome—a statement which cannot be accepted. In Paratelphusa it has been shown that the
axial filament (central body) arises from the proximal centrosome, but it has no connexion whatsoever with the intra-nuclear karyosome which gradually disappears in the course of spermatelosis.

Why are the mitochondria and the Golgi apparatus regarded as such in *Paratelphusa*? Apart from the very strong morphological evidence, into which it is quite unnecessary to enter here, there is the much stronger functional evidence. All cytologists agree that in the spermatogenesis of flagellate sperms the mitochondria form a sheath at least of some part of the axial filament and that the Golgi apparatus is concerned in the formation of the acrosome. The structures described as mitochondria and Golgi apparatus in the spermatid of *Paratelphusa* have exactly the same destiny.

This brings us to the very obvious and the most important conclusion of the present investigation—that the crab spermatozoon, in spite of its weird form, is essentially like a typical flagellate sperm. Like the latter it possesses a nucleus with which is fused the acrosome, and an axial filament, ensheathed by the mitochondrial nebenkern, extends between the proximal and the distal centrosomes.

This is not all. There are very strong reasons for believing that at the time of fertilization the fantastic appearance of the crab sperm changes into that of a typical flagellate spermatozoon. I possess many Bouin-fixed smears of the testis in which a large number of sperms have exploded, probably as the result of pressure (figs. 31 to 34, Pl. 32). In some cases (fig. 32, Pl. 32) the nucleus rounds off; in others (fig. 34, Pl. 32) it becomes irregular; and in still others (figs. 31 and 32, Pl. 32) it is converted into a shallow cup. The proximal centrosome is considerably stretched, and is represented by the thickened proximal portion of the axial filament. The latter is ensheathed by the now stretched, mitochondrial vesicle. The distal centrosome has become triangular, the distal transverse piece of the axial filament lying at the apex of the triangle. The spermatozoon is now exactly like a typical flagellate sperm with respect to all its components except that the axial filament does not here perform any locomotory function.
What causes the spermatozoon to explode at the time of fertilization I am unable to tell. It may be an increase in osmotic pressure within the mitochondrial vesicle. Whatever the cause the nucleus will be pushed into the egg as the result of the explosion.

Closely connected with this is the question of the function of the acrosome in general. The old idea that the acrosome is a perforatorium to enable the sperm head to pierce the egg membrane (Waldeyer) seems to be irreconcilable with the peculiar position and form of the acrosome in certain sperms (see Bowen, 1924), and the ring-like acrosome of the crab Paratelphusa fused with the posteriorly directed margin of the nuclear cup. Unaided by the rotatory movements of a flagellate axial filament the latter cannot possibly perform a boring function. Besides, in the crab, the entry of the nucleus into the egg seems to be ensured by the peculiar mechanism of explosion. What other function is to be ascribed to the acrosome it is difficult to suggest with certainty, but Bowen has suggested an interesting possibility: that the acrosome may represent the 'sperm-receptors' postulated in Lillie's theory of fertilization.

Lastly, the important point is to be noted that in Paratelphusa spinigera the acrosome arises directly from the Golgi elements and is not a secretory product thereof, as is the case in the genesis of many flagellate spermatozoa. In the latter (e.g. insects) a few Golgi elements (the acroblasts) are said to secrete the acrosome, and after performing this function they are sloughed off along with the rest of the Golgi material. This can, perhaps, be explained by the fact that in the crab there is no spinning out of the cytoplasm along a long axial filament, a process which invariably takes place in the spermateleosis of flagellate sperms.

II. Primary Spermatocytes and Spermatogonia.

Originally I had no intention of discussing the structure of these cells. But Fasten has described in the primary spermatocytes of Cancer magister two round, densely staining, chromatoid bodies each surrounded by a clear area. These bodies are distinct from the centrosome, which may often be
double. No such bodies have been discovered in the spermatocytes of Paratelphusa. On the other hand, there exists in the cytoplasm of these cells the typical Golgi-idiosome complex, consisting of four darkly staining Golgi granules embedded in a very distinct idiosomic area (fig. 36, Pl. 32). Two of these granules are big and invariably lie opposite each other, while the others are smaller and are likewise placed. It is possible that in the spermatocytes of Cancer there are only two big Golgi granules which, in spite of the acetic acid used by Fasten, escaped destruction and were described as the chromatoid bodies by him.

Similarly in the spermatogonium (fig. 37, Pl. 32) there is a Golgi-idiosome complex, but it is much smaller than that of the spermatocytes. As a rule it stains as a single, round granule, but after a careful subtraction of the stain it reveals a structure similar to that found in the spermatocyte.

In discussing the mitochondria-like mass, which is really the acrosome, Fasten considers it likely that it consists of chromatin which has diffused out of the nucleus, as 'in the cells under consideration no traces of mitochondria have been observed in the earlier stages of the maturation'. Here again Fasten erred on account of his having used acetic acid, for in the spermatocytes of Paratelphusa the mitochondria can be observed as extremely delicate granules existing throughout the cytoplasm. On the other hand, the technique used by me (Champy and iron-haematoxylin) has failed to bring out any mitochondrial granules in the spermatogonia (fig. 37, Pl. 32).

It will be recalled that in the spermatid of Paratelphusa the Golgi elements have been described as rings and the mitochondria have been compared to soap bubbles. On the other hand, both these substances exist in the form of granules in the spermatocytes. It is clear that at the commencement of spermateleosis the Golgi granules grow in size and become ring-like, thus crowding out the idiosome; and the mitochondrial granules likewise grow into vesicles with very thin walls. The growth of Golgi granules into rings has been described by Nath (1931) in the eggs of Rana, by Nath and Nangia (1931) in the eggs of Ophiocephalus, and by Bhatia and Nath (1931).
in the eggs of Palaemon. Similarly the mitochondrial granules of the spermatogonia grow into vesicles during the growth-period in the spermatogenesis of scorpions (Wilson, 1925) and some insects (e.g. Gatenby, 1917). Indeed the mitochondria are known to be highly plastic bodies (see Lewis and Lewis, 1914 and 1915).

**SUMMARY.**

The most important conclusion of this investigation is that the crab spermatozoon, in spite of its fantastic form, is with respect to its components exactly like a typical sperm. It possesses a cup-shaped nucleus with the margin of which the ring-like acrosome is fused. The mitochondria-like mass of Koltzoff, Binford, and Fasten are the fused Golgi elements destined to form the acrosome. The cavity of the cup is completely filled by the mitochondria vesicle, corresponding to the 'primary vesicle' of Fasten. The mouth of the cup is very efficiently plugged by the ring-like, distal centrosome which is identical with Fasten's 'chromatin ring'. At the bottom of the mitochondrial nebentkern lies the vesicular, proximal centrosome answering to the description of the 'secondary vesicle' of Fasten. Between the two centrosomes runs a thick axial filament (Fasten's central body).

Evidence has been produced that at the time of fertilization the unusual form of the sperm changes into that of a typical one.

In the primary spermatocytes of Paratelphusa spini-gera there are no 'chromatoid bodies' as described by Fasten in similar cells of Cancer. On the other hand, both in the spermatocyte and the spermatogonium of Paratelphusa, there is the typical Golgi-idiosome complex.

**REFERENCES.**

EXPLANATION OF PLATE 32.

Figs. 1-29 are drawn from smears fixed in Champy’s fluid and stained with iron-haematoxylin.

Fig. 30 is drawn from fresh material stained with neutral red and fig. 35 from similar material studied in a drop of normal saline only.

Figs. 31-4 are from smears fixed in Bouin’s fluid and stained with iron-haematoxylin.

Figs. 36 and 37 are from Champy-fixed sections stained with iron-haematoxylin.

All figures except 30 and 35 are drawn with Leitz 15 x B ocular and x 95 objective at the level of the stage of the microscope, giving a magnification of approximately 1,425 times. Figs. 30 and 35 are drawn without scale.

LETTERING.

A., acrosome; A.F., axial filament; C., centrosome; C^1., proximal centrosome; C^2., distal centrosome; G., Golgi element; I., idiosome; M., mitochondria; N., nucleus; N^1., nucleolus; M.V., mitochondrial vesicle.

Further explanation of figures will be found in the text.