

THE SPERMATOOA OF ALLOLOBOPHORA FOETIDA.

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WITH 1 PLATE.

In the spermatozoön of *Allolobophora* we have demonstrated three centrosome-like structures, one at the base of the spine, one at the anterior, and one at the posterior end of the middle-piece. Heretofore we have differentiated these structures at such rare intervals, we could not claim for them any morphological value, but quite recently, by the aid of photography, we have been able to demonstrate these bodies with sufficient constancy to warrant a consideration of their morphological and functional significance. Do these bodies represent merely points of insertion for the spine, middle-piece and tail, comparable to the basal bodies of cilia, or have they a bearing upon the problems of fertilization?

The morphological value of the apical centrosome-like body is enhanced by the fact that a few investigators, Platner (12), Carl Niessing (11), and Field (3), have traced the centrosome of the spermatocytes to the apex of the head of the spermatozoön, and in one case at least, this apical centrosome appears to function in the fertilized egg, as the centrosome of the male attraction-sphere. To this may be added the interesting observations of King (7), who has shown in the egg of *Bufo* that a male aster is formed at the apex of the head of the spermatozoön.

The morphological value of the centrosome-like body in the middle-piece is enhanced by the interpretations of a large number of investigators who have traced the centrosome of the spermatocytes to the middle-piece of the spermatozoön, in many cases this centrosome being identified in the egg, as the centrosome of the male attraction-sphere.

In the middle piece of *Allolobophora*, however, we have demonstrated two centrosome-like bodies instead of one, and in this connection Lenhossék's (8), observations on the spermatogenesis of certain vertebrates are of interest. He identifies two centrosomes in the middle-piece, these having originated by the division of the centrosome of the spermatid. He shows also a centrosome-like body at the apex of the head—his *Akrosoma*—which he claims, however, has no connection with the centro-

some of the spermatid, arising merely as a thickening of the sphere substance. With improved technical methods we hope to be able to identify within the egg the three centrosome-like bodies of the spermatozoön of *Allolobophora* and determine whether any of them function as focal points for astral rays. For the present the only evidence in the egg, indicating that we may expect to find a centrosome in the spine and in the middle-piece is that the cytoplasm of the egg reacts to *both* spine and middle-piece, this reaction being expressed by two morphologically similar structures, the fertilization cone and the sperm aster, these two structures in turn resembling morphologically the asters of the maturation spindles, each of which contains a centrosome.

On several occasions we have called attention to the similarity of the fertilization cone and the male aster, further homologizing these structures to the poles of a spindle. We quote the following from a former paper: "It is impossible to avoid drawing conclusions as to the morphological significance of the resemblance between the male aster and transverse sections through the fertilization cone. The rays and the central aggregation of *Archoplasm* are as pronounced in the one as in the other, suggesting that each end of the head of the sperm—the spine and the middle-piece—produces on the cytoplasm of the egg a like morphological effect. This would indicate that the spine and the middle-piece are of the same substance, though the identity can not be complete, as the cytoplasm does not react to the two structures at the same stage of the development of the egg. . . . The effect produced by the spine is made, however, by a *moving* object (the sperm entering the egg) and we have thus a different shaped aster—a cone shaped aster. Is it possible that this may have any bearing on the opposing interpretations of various authors, some asserting that the anterior end of the head of the sperm produces the male aster, and others, that the posterior end of the head (the middle-piece) produces it?

"If we accept the interpretation of those authors who claim to have traced a part of the aster of the spermatid, to both spine and middle-piece, may we not regard that part of the spermatozoön (including spine, head and middle-piece) as an attenuated spindle, and expect that each end of this spindle will produce a like morphological effect upon the cytoplasm of the egg?" (5) page 605-6.

When the above was written we had not succeeded in establishing the identity of centrosome-like bodies in either spine or middle-piece, though homologizing the spine, head, and middle-piece of the spermatozoön to an attenuated spindle, made this identification very desirable.

If it can be proved that the fertilization cone and male aster are mor-

phologically alike, it would be convenient to designate them as the *anterior* and *posterior male asters*—and it is impossible to resist an attempt to homologize them to similar structures described for other eggs—although we appreciate the danger of making too rigid an application of the phenomena observed in individual cases.

Lillie (10) in his suggestive work on *Unio* describes two asters, the first, which he identifies as the true sperm attraction-sphere, appears and disappears at about the same stage of the egg's development, as the *anterior sperm aster* (the cone) of *Allolobophora*; while the second, his "accessory aster," appears and disappears at about the same period as the *posterior sperm aster* of *Allolobophora*. It is significant, that the sperm aster of *Unio* is "comet shaped," thus resembling the fertilization cone of *Allolobophora*, and it is also significant that it is found, sometimes preceding the head of the sperm. Is it not possible that the male aster and accessory aster of *Unio* correspond to the *anterior* and *posterior sperm asters* of *Allolobophora*, the approximate agreement in the time of their appearance and disappearance being due to the fact that both eggs are fertilized at about the same stage of development?

In *Axolotl*, Fick (2) figures a distinct spherical body at the base of the spine of the spermatozoon, although he does not call it a centrosome. In the egg he sees a cytoplasmic reaction to the head of the spermatozoon, the so-called funnel. Fertilization occurs later in this egg, than is the case in *Allolobophora*—and yet the funnel and the sperm aster of *Axolotl* are undoubtedly homologous to the *anterior* and *posterior sperm asters* of *Allolobophora*.

In the egg of *Allolobophora* fertilization occurs very early, this fact marking the individuality of these two structures, the *anterior male aster* (the cone) appearing at the metaphase of the first maturation spindle, and the *posterior male aster*, after the first polar body is formed—thus separating the two structures by a period of time as well as position. In eggs in which fertilization takes place later, the cytoplasmic reactions to the spine and middle-piece following each other very rapidly, is it not possible that in some cases the *anterior* and *posterior male asters* may be fused or confused?

If we call in evidence the data indicating that division is one of the life expressions of the centrosome, and if we interpret the three small bodies in the spermatozoon of *Allolobophora* as centrosomes, it involves the unauthorized assumption that the centrosome of the spermatid divides, part being destined to the apex of the head and part for the middle-piece of the spermatozoon, these centrosomes being the equivalent of the one centrosome left in the egg after the formation of the

second polar body, and it is an interesting fact that several investigators have observed the division of this centrosome in other eggs.

If, on the contrary, we attribute to these three spermatie structures the value only of basal corpuscles, we still do not escape the centrosome problem, for Lenhossék (9), and Henneguy (6), claim that the basal corpuscles of cilia have their origin in the centrosome.

If they are indeed centrosomes, we must follow their logical implication and admit that they can be placed in evidence for the theory that the centrosome has its stage of activity and its stage of rest, the former represented by the aster, the latter by the so-called naked centrosome. The stage of activity of the spine and middle-piece centrosomes—assuming they are such—has but an ephemeral expression in the egg, and it seems only logical to assume that after this period of activity they may return again to a resting stage. With more exact technical methods, it may be possible to trace them in the egg during the resting stage and this can be assumed also for the egg centrosome. We wish to accentuate these points, as the egg of *Allolobophora* has heretofore given evidence only, in favor of the theory that the centrosomes arise de novo, and are “the expression rather than the cause of cell activity” (4).

This evidence, in brief, is as follows: The complete disappearance of both male and egg attraction-spheres at a definite stage of the egg's development. A lack of decisive evidence that the rays of the male aster focus at any one point in the middle-piece (5), or that the rays of the cone focus at the base of the spine. Further, an inconstancy in both size and form of the egg centrosome at a given stage of the development of the spindle, and a lack of evidence of any division of either egg or sperm aster.

Although the greater part of this evidence is negative, we have no right to ignore it—we may say rather, that the centrosomes of *Allolobophora* present conflicting evidence that demands rigid cross-examination.

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EXPLANATION OF PLATE.

The spermatozoa shown in this plate were collected at different times from spermatophores found in the slime tubes removed from copulating worms. In each case one spermatophore was teased in a drop or two of water, spread on a slide and dried in the air, or by heat from an alcohol flame. The preparations were stained at once with iron haematoxylin and mounted in balsam.¹

For the photos taken at magnifications of 1000 and 660 diameters, a Zeiss apo. 2 mm., immers. lens, 140 apr., was used, with projection ocular 4, (diaphragm at 0) and camera draw demanded for each magnification. For the photos taken at 450 diameters, the Zeiss apo. 4 mm., lens was used, with projection ocular as above.

For convenience we shall designate the three centrosome-like bodies, as the apical granule, and the anterior and posterior granules of the middle-piece. These granules are seen also in spermatozoa found in the spermathecae, but the photo of these was overlooked in preparing the plate.

In the half-tone plate some of the granules were strengthened slightly in order to secure satisfactory printing. If any of our readers should wish to compare the reproduction with the original prints, the latter may be obtained on request.

Photo 1. Spermatozöon, showing spine, head, middle-piece, part of the tail, and the three granules, one between the spine and head, one between the head and middle-piece, and one between the middle-piece and tail. Mag. 1000.

¹ Some of the slides were examined unstained, in glycerine, after five hours' immersion in a saturate solution of osmic acid. The spermatozoa failed to show any osmophile granules.

Photo 2. Spermatozoön from the same spermatophore, though not the same slide as Photo 1. The magnification is less, *i. e.*, 660. The two granules in the middle-piece were not clearly differentiated in this preparation, and we therefore focused on the apical granule. The apical granule is more constantly differentiated than the granules in the middle-piece which require a magnification of one thousand diameters for satisfactory illustration. Photos 3, 4 and 5 further demonstrate this point.

Photo 3. See under Photo 2. Mag. 660.

Photos 4 and 5. Spermatozoa from two spermatophores collected five days apart. Mag. 450. These preparations were photographed to show the constancy of the presence of the apical granule. The lower magnification was used to bring into the field a larger number of spermatozoa than is possible with a 2 mm. lens.

Photo 6. Anterior end of a spermatozoön from the same spermatophore as the spermatozoön shown in Photo 1. On every slide dried in the air, or by heat, there are areas in which entire spermatozoa, or definite parts of them, are much flattened, sometimes the chromatin of the head flowing into a broad, thin layer, in some cases with a line of alveolar cytoplasm on each side of the head, and sometimes the tail splitting into parallel fibres. This is due perhaps to rapid and uneven drying. In such spermatozoa, the three granules are much more clearly differentiated. We have selected a few preparations to illustrate this. In Photo 6, the chromatin of the anterior part of the head is flattened, as described above, and the apical granule sharply differentiated. The effect on the form of the head, produced by the flattening, is seen by comparing the part of the head next the spine, with the part cut by the edge of the photo. Mag. 1000.

Photo 7. Anterior end of a spermatozoön, showing spine and part of head. The head is much flattened and almost completely severed from the apical granule, which is thus sharply differentiated. Mag. 1000.

Photo 8. Spermatozoön showing spine, apical granule and head, the middle-piece with anterior and posterior granules and a part of the tail. The part of the head next the middle-piece is slightly flattened and the tail also is flattened and split into parallel fibres; this condition of the head and tail allowing a sharp differentiation of the two granules in the middle-piece. Mag. 1000.

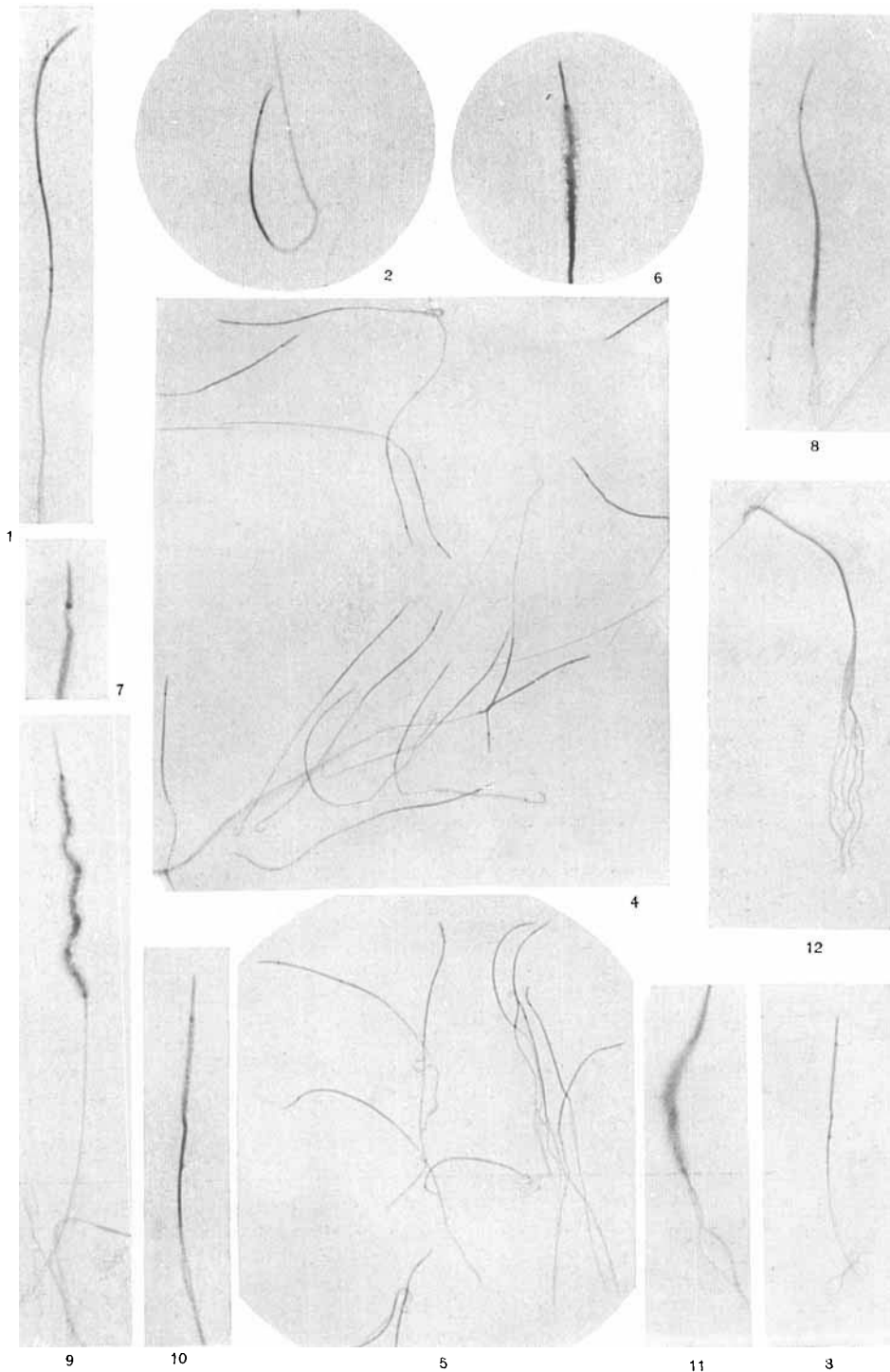
Photo 9. Spermatozoön with flattened head, showing apical granule and the two granules of the middle-piece. *C. f.* Photo 6. Mag. 1000.

Photo 10. Spermatozoön, showing spine, apical granule, head with anterior half slightly flattened, and part of the tail. Mag. 1000.

Photo 11. Part of the head of a spermatozoön much flattened; middle-piece showing anterior and posterior granules, and part of the tail split into parallel fibres. Mag. 1000.

The spine with apical granule was not included in this photo, because not on the same plane with the middle-piece, and thus requiring a different focus.

Photo 12. This spermatozoön was photographed to show the fibrillar structure of the tail, this splitting of the cytoplasm of the tail being due



probably, as stated above, to some unusual condition in the drying on the slide, for it occurs only in definite areas. Mag. 660. The head of the sperm is slightly out of focus, for it was necessary to sacrifice this detail, to get an exact focus on the extremely fine fibrillae of the tail.

This preparation resembles many of the figures Ballowitz gives of the spermatozoa of Coleoptera (1).