THE SPERM CENTROSOME AND ASTER OF ALLOLOBOPHORA FOETIDA.

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WITH 1 PLATE.

During the past few years evidence has accumulated which assigns to the egg attraction-sphere a position where it threatens to usurp all the functions heretofore claimed for the male attraction-sphere. This promotion of the egg centrosome and aster with its satellites the cytasters, seems to have been at the expense of the male centrosome, until even Boveri can suggest as a possible hypothesis "Anstatt wie bisher zu sagen das Spermatozoon führt ein Centrosoma ins Ei ein, müsste es heissen: das Spermatozoon bewirkt im Ei die Bildung eines Centrosoma, aus dessen Teilung alle folgenden hervorgehen." (Das Problem der Befrüchtung. Theodor Boveri, Jena, 1902.)

The egg of *Allolobophora* furnishes evidence which indicates that the centrosome of its male attraction-sphere is part of the spermatozoön itself, this evidence supporting the interpretation of Boveri and others, who maintain that the sperm centrosome is carried into the egg by the spermatozoön.

In 1902[1] we demonstrated the constant presence of three granules in the spermatozoön of *Allolobophora;* one between the spine and head, one between the head and middle-piece, and one between the middle-piece and tail. Photographs of spermatozoa showing these granules can be seen in the above-mentioned article.

It has been our aim to determine whether either granule at each end of the middle-piece forms the morphological center of the male attraction-sphere, or whether the entire middle-piece must be regarded as the morphological centre. In every case in which we have found the middle-piece attached to the head, and its relation to the aster-rays could be therefore accurately demonstrated (photos. 1-8), it is certain that the

[1] The Spermatozoa of Allolobophora foetida. The American Journal of Anatomy, Vol. I, No. 3, 1902.

posterior granule of the middle-piece functions morphologically as a centrosome. The morphological value of this fact is, however, challenged by Van der Stricht's interesting results in his study of the egg of the bat, where he finds the rays of the sperm aster focussed around the *anterior* granule of the middle-piece. " Un spermaster se forme tout autour d'un corpuscle central, le spermocentre, attenant à l'extrémité antérieure de la pièce de réunion de la queue du spermatozoïde." [2]

The origin of the sperm centrosome in Allolobophora has been very difficult to determine, as preparations showing the exact stage of development necessary to decide this point are rarely found. The middle-piece must be not only intact, but it must be still attached to the head of the spermatozoön; for after it is separated from the head its position within the sphere (photo. 9) is most erratic. Its length may be at any angle in relation to the head of the spermatozoön, and frequently the whole middle-piece is entirely out of the centre of the sphere. This is the stage of the male attraction-sphere usually found; and this fact made it impossible to assign to either of the granules of the middle-piece the morphological value which now seems warranted by our recent preparations.

It is probable that this displacement of the middle-piece in the sphere is due to the mechanical effect of the fixatives. The fixatives and the subsequent technique shrink these eggs, in many cases, one-third of their diameter, and this fact indicates that we may expect a displacement of the structures, unless we assume that the forces act equally on all the constituent parts. In a former paper [3] we have shown photographs of different cytological configurations produced in these eggs by the various fixatives, and the displacement of cytological constituents has been demonstrated by many investigators. We have nearly a thousand photographs systematized in such a way as to facilitate a comparative study of the effect of the different fixatives at definite stages of the egg's development; but the inconstancy of the reactions of the egg to the fixative under conditions apparently the same, makes the problem too complicated to justify hasty conclusions.

Photos. 1 to 8 show the middle-piece intact and still attached to the head of the spermatozoön, while the sphere is formed around the *posterior* end of the middle-piece. In photos. 1, 4, 6, and 8, the posterior granule itself is clearly seen. That these granules are larger than those in the spermatozoön before it enters the egg—though perhaps due in part to

[2] Van der Stricht, O. Le spermatozoïde dans l'oeuf de chauve-souris (V. noctula). Verhand. d. Anat. Gesell., 1902.

[3] Photographs of the egg of Allolobophora foetida. Journ. Morph., Vol. XVI, No. 3, 1900.

fixation—is in keeping with the fact that the spermatozoön increases in size as soon as it enters the egg, the head becoming longer and broader, before it begins to contract into the short thick rod of later stages. This separation of the head and middle-piece appears to be caused by the contraction of the head rather than by the migration of the middle-piece and sphere. The separation of the contracted rod from the middle-piece (photo. 9) is no greater than the contraction of the head would necessitate.

In photograph 9, although the middle-piece is separated from the head, its posterior end—the one farthest from the head—is almost exactly in the centre of the sphere.

Photograph 10 shows the male attraction-sphere and a cross-section through the posterior end of the middle-piece, with the tail of the spermatozoön still attached to the posterior granule. The artificial appearance of the tail in this section (probably due to the fixative) would make us hesitate to interpret this structure as the tail of the spermatozoön, if we had not often found the tail persisting until this stage, in eggs killed in other fixatives. Photographs of some of these preparations were shown in an earlier paper.[4] Photo. 3 also shows an indication of the persistence of the tail; the middle-piece being still attached to both head and tail, its posterior granule forming the center of the sphere.

In photo. 7, part of the head of the spermatozoön and its middle-piece are clearly defined, and the rays of the aster focus at the posterior end of the middle-piece. The posterior granule of the middle-piece is obliterated by over-staining.

The demonstration of the morphological value of this granule encourages us to hope that we shall find preparations showing that the acrosome forms the focal point for the first rays of the fertilization-cone, thus warranting our assumption of a morphological value to this centrosome-like granule, and supporting our interpretation of the morphological similarity of the male attraction-sphere and the fertilization-cone.

Although we are forced to the conclusion that these preparations indicate that a definite part of the spermatozoön itself forms the centrosome of the male attraction-sphere, *Allolobophora* fails to offer any evidence that this centrosome gives rise to one or both of the cleavage centrosomes. At a later stage the entire middle-piece disintegrates into several granules[5] and disappears completely, and there is no proof that the granule which persists the longest is the posterior granule of the middle-piece.

[4] Photographs of the egg of Allolobophora foetida. Journ. Morph., Vol. XVI, No. 3, 1900.

[5] Photographs of the egg of Allolobophora foetida. Journ. Morph., Vol. XVI, No. 3, 1900.

We might assume this if one of the granules could be traced to the cleavage stage, but at the telophase of the second maturation-division, both egg and sperm-attraction spheres, with their centrosomes, disappear. Until improved technique enables us to trace the centrosome through these stages, the evidence given by *Allolobophora* still points to the *de novo* origin of the cleavage centrosomes.

EXPLANATION OF PLATE I.

The photographs of this plate were taken by the method described in full in Zeit. f. wiss. Mik., Bd. XVIII, 1901. "A new method of focussing in photomicrography."—Foot and Strobell.

In order to economize space only a small part of each section is shown in the photographs.

All the sections were stained with iron hæmatoxylin, in most cases with an after stain of dilute Bismark brown.

PHOTO. 1. Section (3μ) of oöcyte, second order, showing the male attraction-sphere with the posterior granule of the middle-piece forming the center of the sphere. The middle-piece of the spermatozoön is intact and still attached to the head, part of which appears in this section, the rest of the head showing in four neighboring sections. Fixative Perenyi's fluid. × 1000.

PHOTO. 2. Section ($2\frac{1}{2}\mu$) of oöcyte, second order, showing the male attraction-sphere with the posterior end of the middle-piece nearly in the center of the sphere. The middle-piece of the spermatozoön is intact and still attached to the head, part of which shows in this section. The rest of the head in three neighboring sections. Fixative Hermann's fluid. × 1000.

PHOTO. 3. Section (3) of oöcyte, second order, showing male attraction-sphere with posterior end of middle-piece in the center of the sphere. The middle-piece is intact and still attached to both the tail and head of the spermatozoön. The rest of the head is in the next section. Fixative corrosive sublimate. × 1040.

PHOTO. 4. Section (3μ) of oöcyte, second order, showing male attraction-sphere with the posterior granule of the middle-piece demonstrated in the center of the sphere. The middle-piece of the spermatozoön is intact, and is still attached to the head, a part of which appears in this section. The rest of the head is in the next section. Fixative Perenyi's fluid. × 1000.

PHOTO. 5. Section (3μ) of oöcyte, second order, showing male attraction-sphere with posterior end of middle-piece in the center of the sphere. The middle-piece of the spermatozoön is intact and still attached to the head, a part of which appears in this section. The rest of the head in the two following sections. Fixative corrosive sublimate. × 1000.

PHOTO. 6. Section (3μ) of oöcyte, second order, showing male attraction-sphere with the posterior granule of the middle-piece demonstrated in the center of the sphere. The middle-piece of the spermatozoön is intact and still attached to the head, a part of which appears in this section; the rest of the head in three neighboring sections. Fixative corrosive sublimate. × 1000.

PHOTO. 7. Section (2½μ) of oöcyte, second order, showing male attraction-sphere with posterior end of middle-piece in the center of the sphere (overstaining has obliterated the granule). The middle-piece is intact and still attached to the head, part of which appears in this section. The rest of the head is in two neighboring sections. Fixative chromo-acetic. × 1000.

PHOTO. 8. Section (3μ) oöcyte, second order, showing male attraction-sphere with posterior granule of middle-piece in the center of the sphere. The middle-piece is intact, and still attached to the head, part of which appears in this section. The rest of the head is in three neighboring sections. Fixative corrosive sublimate. × 1040.

PHOTO. 9. Section (2½μ) of oöcyte, second order, showing male attraction-sphere with posterior end of middle-piece in the center of the sphere. The middle-piece is intact, but separated from the head, part of which shows in this section. The rest of the head is in the next section. Fixative corrosive sublimate. × 1000.

PHOTO. 10. Section (3μ) of polysperm oöcyte, second order, showing one of the male attraction-spheres and part of the tail of the spermatozoön attached to a granule, which must be the posterior granule of the middle-piece. Fixative Hermann's fluid. × 1000.

PHOTO. 11. Section (2μ) of oöcyte, second order, showing male attraction-sphere with one end of middle-piece in center of the sphere. The rest of the middle-piece shows in the next section as a tiny rod with the opposite end near the periphery of the sphere. Fixative Flemming's fluid without acetic acid, × 1000.

PHOTO. 12. Section (2μ) of oöcyte, second order, showing male attraction-sphere with middle-piece, one end of which is in the center of the sphere. The end at periphery of sphere is nearest the head of spermatozoön, which is in neighboring sections. Fixative Flemming's fluid, without acetic acid. × 1000.

SPERM CENTROSOME OF ALLOLOBOPHORA FOETIDA. PLATE I.

FOOT & STROBELL PHOTOS.