THE ORIGIN OF THE CLEAVAGE CENTROSOMES.

KATHARINE FOOT, Evanston, Illinois.

DURING the past two years the weight of authority has greatly increased in favor of the conclusion that the cleavage centrosomes are of spermatogenic origin.

These centrosomes are asserted to be the daughter centrosomes of the so-called male centrosome, and this is assumed to originate from the middlepiece of the spermatozoon, — to be of the very substance of which the middlepiece was formed.

The phenomena of fertilization in the egg of *Allolobophora foetida* do not sustain this view; they suggest that this centrosome has the same origin as the so-called egg centrosome, and that both are cytoplasmic elements, of like origin and constitution.

The sperm attraction sphere and the fertilization cone have several points in common; both structures appear to be dependent, not alone upon the entrance of the spermatozoon, but also upon a definite stage of maturation reached by the egg; for I have never found a cone after the first polar body is constricted off, nor a sperm attraction sphere before the anaphase of the first spindle, no matter how far the spermatozoon may have penetrated into the egg; both appear to be formed of substances not confined to the structures themselves, but belonging to the entire cytoplasm.¹

These observations suggest that both the cone and the attraction sphere are expressions of a definite effect produced upon the cytoplasm by the entrance of the sperm, the phenom-

¹ In the summer of 1895 I differentiated from the cytoplasmic network a substance (archoplasm) which is present in the attraction spheres, in the fertilization cone, in the spindle, and throughout the cytoplasm (see *Journ. of Morph.*, vol. XII, Plate I). Recently I have differentiated the microsomes and centrosomes from the cytoplasm and archoplasm (Figs. 2, 3, 4, 7, 9, 10). These microsomes do not appear to be merely thickenings of the cytoplasmic threads; they appear to be morphological elements, many of them scattered throughout the cytoplasm in a relatively independent manner, others imbedded in the cytoplasmic network.

ena differing in that the anterior end of the head of the spermatozoön produces a cone, whereas it is the middlepiece that produces the attraction sphere.

Before the first polar body is formed we find a cone the moment any part of the head of the spermatozoön penetrates the egg, and the extent of the cone appears to depend upon the length of head which has entered: if a relatively short piece of the head has entered, we find a relatively small cone. The sperm attraction sphere, on the contrary, does not appear until the middlepiece is within the egg, and in other forms this fact has been cited as evidence for the assumption that the centrosome of the sperm attraction sphere is of the substance of which the middlepiece of the spermatozoön was formed.

The more important observations adduced to prove that the male centrosome is formed from the middlepiece are the following: First, the attraction sphere does not appear until after the spermatozoön has entered the egg. Secondly, it appears at or near the point formerly occupied by the middlepiece of the spermatozoön. Thirdly, the middlepiece and the centrosome have been shown to select the same stains. Might not the first and second observations be cited with equal pertinence to prove that the spermatozoön produces merely a physiological *effect* upon the cytoplasm? The third observation has a more definite bearing upon the point in question. In the egg of *Allolobophora foetida*, however, I have differentiated by two methods the middlepiece of the spermatozoön from the male centrosome. This differential staining might be explained by the assumption that the middlepiece undergoes a physiological change on entering the egg; but as the head gives no such evidence of physiological change, are we justified in assuming without further evidence that this takes place in the middlepiece?

The centrosomes of the egg of *Allolobophora foetida* lend support to the view that the centrosome is a mechanical center — the *expression*, rather than the cause, of cell activity. The egg attraction sphere is present during the two maturation divisions, but after the second polar body is formed and the female pronucleus begins to develop, it totally disappears (Fig.

5). The sperm attraction sphere is present until the head of the spermatozoon begins to develop into the male pronucleus, when it also totally disappears (Fig. 6). Both spheres are absent during a relatively long period (*i.e.*, while the young pronuclei are developing); and when the pronuclei have attained their maximum size and are in contact, two attraction spheres appear again in the cytoplasm, and the cleavage spindle is formed.

Method.—The middlepiece of the spermatozoon was first differentiated from the centrosomes by iron haematoxylin followed by erythrosin. The same results were obtained by staining with a mixture of orange and methyl green. (Saturated aqueous solution of orange two parts, in eight parts water. Saturated aqueous solution of methyl green one part, in four parts water. Wash in absolute alcohol.) This stain was the outcome of a series of experiments with the anilins, undertaken with the aim of supporting the method of double staining (Figs. 8–10) by one containing fewer possibilities of error. This method demands relatively no technical manipulation, and practically the same results were obtained whether the sections remained in the stain fifteen minutes or twenty-four hours. The stain was used after several fixatives, with the same result, but all the figures for this paper were drawn from chromo-acetic preparations.

An examination of the figures will show that a variety of structures select the methyl green, — *viz.*, the nucleoli, microsomes, centrosomes, chromosomes, and sperm granules, — and that the orange is selected by the chromatin of the vesicles and resting nucleus, the cytoplasmic network, and the archoplasm (which is present in the attraction spheres, fertilization cone, spindle, and throughout the cytoplasm).

This method of staining sharply differentiates the very young nucleoli of the vesicles found at the telophase of the second maturation spindle, and destined to become the female pronucleus. These vesicles when first formed are eleven in number, thus corresponding with the chromosomes. As they progress towards the formation of the female pronucleus, their walls break and fuse with each other, thus forming the chro-

matic network of the pronucleus, while their nucleoli grow and fuse with one another. Fig. 5 shows several small vesicles and a few relatively large vesicles, the result of the fusing of two or more small ones. Just before the vesicles appear, the chromosomes select the methyl green stain, but as soon as the vesicles are formed we have a tiny, green nucleolus surrounded by *yellow* chromatin, this clearly suggesting that the nucleolus is a substance which a moment before was contained in the chromosomes. The chromatin which at first surrounds each vesicle continues to select the orange stain until it has again assumed the form of chromosomes. Fig. 6 shows the male pronucleus forming in exactly the same manner as the female pronucleus, the head of the spermatozoön breaking up into vesicles similar to those of the young female pronucleus.

Vesicles entirely similar to those found at the telophase of the second maturation spindle are found also at the telophase of the first maturation spindle, though I have never seen any evidence of their development into a resting nucleus.

Sperm Granules, Fig. 2. — The sperm granules are not constant structures. In eggs found during the height of the breeding season (when they are less likely to present abnormalities), the sperm granules are either not present at all or are relatively insignificant in both size and number, and in such cases they are often found near the posterior end of the head of the spermatozoön. Near the close of the breeding season, however, when few normal eggs are found (sometimes only one in a cocoon that contains fifty eggs, showing various degrees of degeneration), — at this season nearly all the eggs having one or more fertilization cones contain relatively many and large sperm granules. These granules appear to be formed at the expense of some of the surrounding substance, and such preparations as are represented by Fig. 2 suggest that the substance sacrificed to the formation of these bodies is archoplasm, for there is relatively very little archoplasm at the side of the cone occupied by the sperm granules.

The suggestion that these granules are metamorphosed archoplasm raises several questions that I am entirely incompetent to answer. Wherein do they differ from apparently

similar, and also inconstant, large and small granules (or bodies) found outside and sometimes within the first and second maturation spindles (Fig. 4), outside and sometimes within the attraction spheres (Figs. 3, 4, 9, 10), and, again, often scattered throughout the cytoplasm? Furthermore, as no exact distinction can be made as to size, how do these bodies differ from the microsomes? (Figs. 2, 3, 4, 7, 9, 10.) Can these sperm granules and large granules throughout the cytoplasm — apparently indicative of incipient degeneration — be at first merely an expression of abnormal activity in the cell? These questions must be left to the more experienced student to answer.

I am greatly indebted to Dr. Whitman for criticism of my work, and for other courtesies in connection with this paper.

EXPLANATION OF PLATE XXXIX.

Zeiss hom. immer. 2 mm. 140 ap. Abbe Camera.

FIGS. 1-7. Orange, methyl green. See Method, p. 811.

FIGS. 8-10. Iron haematoxylin followed by erythrosin.

FIGS. 1, 2, 3, 4, 8, $\times$ about 687.

FIGS. 5, 6, 7, 9, 10, $\times$ about 500.

FIG. 1. Spermatozoön. (Middlepiece yellow.)

FIG. 2. Longitudinal section through fertilization cone. (Only such microsomes represented as appear to be independent of the cytoplasmic threads.)

FIG. 3. Sperm rod and sperm attraction sphere. (Microsomes and centrosome green.)

FIG. 4. Longitudinal section through second maturation spindle.

FIG. 5. Section of egg. (Telophase of the second maturation spindle. Vesicles forming female pronucleus. Egg attraction sphere has nearly disappeared. Microsomes not represented.)

FIG. 6. Vesicles forming male pronucleus. (Male attraction sphere has disappeared. Microsomes not represented.)

FIG. 7. Pronuclei in contact. (Attraction spheres present.)

FIG. 8. Spermatozoön. (Middlepiece red.)

FIG. 9. Male attraction sphere. (Microsomes and centrosome black.)

FIG. 10. Egg attraction sphere. (Microsomes and centrosome black.)