PHOTOGRAPHS OF THE EGG OF ALLOLOBOPHORA FOETIDA.

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I.

THIS paper is the first of a series in which we hope to make a careful comparative study—illustrated with photographs—of the effects of various fixatives on the cytoplasm of the egg of *Allolobophora foetida*.¹

In addition a number of preparations have been photographed to illustrate the following points:

The morphological resemblance of the fertilization cone to the male aster.

The position of the middle-piece in the male aster.

The origin of the sperm granules.

The early stages of the development of the pronuclei.

The presence of osmophile granules in the nucleoli of the germinal vesicles.

The photographs have been taken at only two magnifications (660 and 950). No photographic feats have been attempted, photography being used merely to register the points we wish to illustrate, and the higher magnification has not been used where our aim could be achieved by the lower.

Cytoplasmic Reaction to Fixatives.

Our aim is to make a comparative study of the effect produced not only by the compound fixatives, but by the component parts of each. If with a given fixative we find a definite cytoplasmic configuration, can this be materially changed by omitting one of the constituents of the fixative? If this

¹ A few photographic illustrations of this comparative work were published in 1898. Foot and Strobell, "Further Notes on the Egg of *Allolobophora foetida*," *Zoological Bulletin*, vol. ii, No. 3.

proves to be the case, will this constituent produce a like effect in combination with another fixative? We hope by a careful comparative study to be able to determine how much of the structure seen in fixed cytoplasm is due to the fixation.

The cytoplasm reacts very differently to different fixatives; for example, the spaces occupied by the hyalin globules¹ are in some cases distinctly defined (photo. 17), while in others the globules have apparently fused in all directions, producing a scattering of the intermediary substance and consequent formation of rays.

It is a significant fact that in those preparations where the spherical spaces occupied by the hyalin globules are destroyed, we usually find more definite rays, this being clearly shown by comparing photo. 17 with photos. 15, 19, 21, and 22 (these five photos. showing nearly the same stage of development of the egg).

The last four, with many of the chromo-acetic preparations (*e.g.*, 2, 8 and 9), might be called in evidence for the reticular theory of cytoplasm, and photo. 17 supports with equal force the alveolar theory.

We are convinced that the indication of rays we have seen in the *living* eggs is not comparable to the rays seen in preparations where the hyalin globules have fused and scattered.

Twelve fixatives are represented in our three plates, but we do not wish to assert that any one of these preparations represents the typical effect of the special fixative, the reaction of the egg to any one fixative being very inconstant. This complicates the problem and a comparative study can be profitably carried out only by an exhaustive collection of photographs. In the present paper attention will be called to a few suggestive comparisons, awaiting further data before attempting to draw definite conclusions.

¹ At Dr. Whitman's suggestion we have adopted the term hyalin globules to designate the substance which in an earlier paper we called sap globules. This substance, which is in globular form only at certain stages, we interpret as synonymous with the hyaloplasm of some authors. We retain the term globule, because when this substance is pressed out of the living egg, it keeps its globular form, not fusing with water for several minutes. Treatment with osmic acid has failed to demonstrate any fatty constituent.

*Archoplasm in the Male Attraction Sphere.*¹

Sometimes the archoplasm is quite uniformly distributed, and again it is represented by rods or rays or granular masses. As these varying forms are seen at the same stage of development of the egg, is it not probable that they represent varying expressions of the effect of the fixatives?

An examination of the photos. of eggs killed in corrosive-sublimate (12, 14, 15, 18, 20) reveals a marked similarity in the distribution of the archoplasm. It forms a granular, flocky circle around the male attraction sphere, and this arrangement is relatively constant for corrosive-sublimate preparations.

A comparison of these with photo. 17 shows a striking difference, and we are forced to conclude that the approximately even distribution of archoplasm in this section is more suggestive of the living condition, because the hyalin globules have not fused, as is probably the case in the corrosive-sublimate preparations, and the action of the fixative must be thus less injurious. In the above list of corrosive-sublimate preparations we did not include photo. 16, for the arrangement of the archoplasm of that section is exceptional—it is more like that shown in photo. 17, and it is a significant fact that the fixation of the rest of the cytoplasm is also more like that of photo. 17, *i.e.*, there has been less fusing of the hyalin globules. The egg of photo. 17 was fixed with Hermann's fluid, *without* acetic acid; the same stage fixed with Hermann, in which the acetic acid had been retained, shows a very different cytoplasmic configuration.

The archoplasm of photo. 21 (Perenyi's fluid) and of 19 (Flemming's fluid strong) is aggregated into decided rays, and there are no indications of the presence of hyalin globules.

The archoplasm of photo. 7 is more condensed than is the case in any of the other preparations. The fixative used (osmic

¹ We are preparing a paper to demonstrate (with a series of photographs) the presence of the archoplasm throughout the cytoplasm and its homology to the so-called yolk-nucleus. We hope to defend this broader use of the term, employed in an earlier paper, and to support the interpretations there suggested. Foot, "Yolk-Nucleus and Polar Rings," *Journ. of Morph.*, vol. xii, No. 1, 1896.

and acetic) shrunk the egg nearly one-half its diameter, and the archoplasm is shrunken into compact masses that resemble rods.

The archoplasm of photo. 9 (chromo-acetic) forms a sharp contrast to that of photo. 7. Part of it is aggregated at the center of the attraction sphere, where it is stained so deeply that the middle-piece is completely obliterated.

Fertilization Cone.

Photo. 1 shows the size of the cone in relation to the entire egg (at this stage the first maturation spindle is in the metaphase and at the periphery of the egg). Photo. 2 is a section of such an egg, cut longitudinally through the cone. The thinness of the section enables us to see the head of the sperm within the cone, on its way to the center of the egg.¹ The sperm continues its course until its middle-piece is near the inner aster of the first maturation spindle, slightly turning and bringing its head again near the periphery, some of the preparations (for example, photo. 12) producing the false impression that it has entered from the latter point, and the aster has formed at the apex of the head. The progress of the sperm towards the inner aster of the spindle appears to be dependent upon the stage of development of the egg. When the spindle has reached the anaphase, the sperm aster is formed and the progress of the sperm towards the center of the egg then ceases, the head separates from the middle-piece and contracts into a short thick rod (photos. 8, 16, 17, and 18). The spiral twist of the sperm shown in photo. 2 is an uncommon form, but it probably does not indicate an abnormal condition of the egg.

In photo. 5 we have an unmistakably pathological cone, this enabling us to determine the pathological condition of the rest of the cytoplasm and giving a standard of comparison which may prove of service.

Photo. 6 probably represents another example of pathological cytoplasm, as the egg contains four spermatozoa, two of which are shown in the section photographed. It is a question

¹ Over-printing at the apex of the cone has obliterated the sperm at that point.

how much of the difference in the cytoplasmic configuration of photos. 5 and 6 is due to the difference in fixation, the egg of photo. 5 having been killed in corrosive-sublimate and that of photo. 6 in picro-formalin. Photo. 6 represents, however, a little later stage of development of the egg.

Morphological Similarity of the Cone and Male Aster.

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.

Photo. 3 shows a transverse section of a fertilization cone, near its apex, and a comparison of this with a section through the male aster of photo. 9 will serve to illustrate this point. 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 (see photo. 37) 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 cannot be complete, as the cytoplasm does not react to the two structures at the same stage of development of the egg. There are a few investigators who claim to have traced a substance in the spermatid to both spine and middle-piece. 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,¹ and expect that each end of this spindle

¹ Foot, "The Centrosomes of the Fertilized Egg of *Allolobophora foetida*," *Biol. Lect., Marine Biological Laboratory.* Boston, 1896.

will produce a like morphological effect upon the cytoplasm of the egg? We have been unable to differentiate in either spine or middle-piece any special structure that we feel justified in interpreting as a centrosome.

Further Observations on the Middle-Piece of the Sperm and its Morphological Rôle in the Male Aster.

In 1897¹ one of us differentiated in color the centrosome of the male aster from the middle-piece of the spermatozoön, this leading to the interpretation that the centrosome of the male aster is of purely cytoplasmic origin—the middle-piece merely producing the cytoplasmic phenomenon known as the aster, but no definite part of the sphere being formed of the middle-piece substance.

Further investigation has demonstrated that the middle-piece can remain for a definite period intact within the aster, and that the later differentiation in color is probably due to chemical change, for it disintegrates before disappearing.

The photos. of Pl. XXXVI show the middle-piece within the aster. Although it is by no means always in the center, we have been able to find no other structure that we feel justified in interpreting as a centrosome.

The middle-piece finally disintegrates and totally disappears, and there is no evidence that it takes any part in forming the cleavage centrosomes. As stated in the paper above referred to (I), during the formation of the young pronuclei both the egg and sperm centrosomes totally disappear, and there is no evidence that either takes part in forming the cleavage centrosomes, this egg supporting the theory that the cleavage centrosomes arise *de novo* in the cytoplasm.

In photo. 12 we see a portion of the head of the spermatozoön, its middle-piece within the aster, and a part of the tail (the missing part of the head is in the next section). The head at this stage shows constrictions at definite intervals (these are lost in some of the reproductions of photo. 12, but

¹ Foot, "The Origin of the Cleavage Centrosomes," *Journ. of Morph.*, vol. xii, No. 3, 1897.

two are shown in photo. 14). We are unable at present to homologize these divisions of the sperm head with the chromosomes of the egg, not having seen the requisite number.

If the archoplasm shown at the periphery of this sphere is comparable to Boveri's archoplasm in ascaris, it certainly is not brought in by the sperm, as claimed by several authors for the ascaris egg, for here we see the spermatozoön still intact.

Photo. 13 shows part of the head of the sperm and the entire middle-piece, at about the same stage as that of photo. 12, though the magnification is somewhat greater (950). Without more data we hesitate to interpret the two tiny filaments at the posterior end of the middle-piece. One of them may be the proximal end of the tail of the spermatozoön, or they may both represent the splitting of the proximal part of the tail and its fusing with the cytoplasm of the egg. This egg was killed in 2 per cent osmic in 70° alcohol, and it is interesting to compare its cytoplasmic structure with that of photo. 12, in which case the egg was killed in corrosive-sublimate.

In photo. 14 we have the same stage of development of both sperm and middle-piece.

In photo. 15 we have transverse sections of two middle-pieces within the aster, and it is scarcely necessary to call attention to their resemblance to a dividing centrosome. The heads of the two spermatozoa are in the adjacent sections. They are contracted rods such as those shown in photos. 16, 17, and 18.

Photo. 16 shows the head of the spermatozoön separated from the middle-piece and contracted into a relatively thick short rod — the middle-piece has (apparently) contracted somewhat, and we interpret the filament to the right of the middle-piece as a part of the tail of the spermatozoön.

In the preparation represented by photo. 17 there is a middle-piece distinctly seen within the sphere; but it has been obliterated by too dark printing in the reproduction, over-printing producing the same results as over-staining.

In photo. 18 the middle-piece is by no means in the center of the sphere; but the spherical form of the latter remains intact. If the middle-piece does more than stimulate the egg

to the expression of cell activity known as the male attraction sphere, if it is the organic center of the sphere, forming with the rays an organic connected whole, would not the spherical form of the sphere be disturbed by the middle-piece moving away from the center? In reality the form is no more disturbed than is the form of the fertilization cone, when we find the head of the spermatozoön quite out of the center of the cone.

In photo. 19 the middle-piece is in nearly the center of the sphere. (The contracted head of the sperm is in the adjacent sections.)

In photo. 20 we have a transverse cut through the middle-piece, which bears a marked resemblance to a centrosome, though its position in the sphere is eccentric; the spherical form of the sphere, however, remains undisturbed. (The contracted head of the sperm is in the next section.)

Photo. 21 shows a little later stage of development than that of 18; the middle-piece is beginning to disintegrate, and at a still later stage it has entirely disappeared. The differentiation in color which one of us obtained between the middle-piece of the spermatozoön and a distinct spherical body within the male aster was probably due to the chemical change which must take place at this time of disintegration.

Photo. 22 shows about the same stage of development as that of photo. 21; the middle-piece is beginning to disintegrate, showing three tiny spherical bodies. The middle-piece and rays finally disappear at about the same time.

Further Observations on the Origin of the Sperm Granules.

Photos. 10 and 11 indicate an origin of the pathological sperm granules different from that suggested by one of us in 1897.¹

They were interpreted then as being formed at the expense of the archoplasm, for the reason that in those cases where they were present in the cone they were surrounded by an area free from archoplasm, indicating that they arose by a concentration of the archoplasm substance at that point.

¹ Foot, "The Origin of the Cleavage Centrosome," *Journ. of Morph.*, vol. xii, No. 3, 1897.

We should expect to find them formed at the expense of some substance in the cell, as they are not constant structures and appear to be a pathological feature. In photo. 10 we have an exaggerated expression of the phenomenon (an unusual number of granules), this aiding us in interpreting their origin. This photograph indicates that they have arisen at the expense of the head of the sperm itself. Less than half of the length of the head is shown in this section; but a comparison of its diameter with that shown in photo. 2 indicates a great loss of substance.

In photo. 11 we see a part of a contracted head of a sperm which appears to have been fixed in the act of constricting off a sperm granule.¹

Early Stages of Development of the Pronuclei.

The photographs of Plate XXXVII illustrate the early stages of development of the male and female pronuclei — four fixatives being represented.

Photo. 23 shows a transverse view through the second maturation spindle, which has reached the anaphase of development. These are the chromosomes approaching the inner pole of the spindle, and which are destined to form the female pronucleus. There are eleven chromosomes in the first and second spindles, and in this photo. each one of the eleven is shown. This photo. was taken by Dr. Fuller, from a slightly crushed oöcyte second order.

Photo. 24 shows a little later stage of development (telophase). This section contains five or six of the chromosomes,

¹ In the *Journ. of Morph.*, vol. xvi, No. 1, 1899, Byrnes describes small round bodies often accompanying the sperm-nucleus in *Limax agrestis*, and says they may owe their origin — although she does not illustrate this point — to particles of chromatin constricted off from the sperm-nucleus before it becomes vesicular, having seen a "few cases in which a portion of the chromatin seemed to be in process of constricting from the sperm head." As she does not suggest they are a pathological expression, on the contrary implying they have a function, it is a question whether they are the same structures we show in photos. 11 and 12. We regret the necessity of mentioning Miss Byrnes's paper in a footnote. The number of the journal in which it appeared was issued in June, 1900, and her paper was not read until just before the receipt of our final proofs.

which have reached the inner pole of the second spindle, the rays of the aster still persisting. A comparison with photo. 23 indicates that these ring chromosomes have been formed by the uniting of the free ends of V-shaped chromosomes, such as those shown in photo. 23.

Photo. 25 shows two (of the eleven) chromosomes at a little later stage of development. Besides the ring, a tiny spherical body appears in connection with each chromosome, and we interpret these as the first appearance of the nucleoli of the female pronucleus, the periphery of each ring representing the chromatin of the chromosomes.¹ If this interpretation is correct, then the periphery of the vesicles which characterize the *later* stages must be interpreted as chromatin, and the spherical body in connection with each, as nucleolar substance. These later stages are shown in photo. 26 (where three of the eleven vesicles are represented) and in photos. 27, 28, 29, 30, 31, and 32. In photo. 27 one of the vesicles shows thick threads of chromatin connecting the nucleolus with the periphery of the vesicle.

Photo. 33 shows part of the head of the spermatozoön breaking up into similar vesicles — the periphery of the vesicle upon which we have focussed we interpret as of chromatin, and the tiny spherical body as nucleolus.

Photo. 34 shows a later stage of the development of the spermatozoön into the male pronucleus, several of the vesicles having fused into one, the rest being in adjacent sections.

Osmophile Granules in the Nucleoli.

It is exceptional to find osmophile granules in the nucleoli, and we are therefore inclined to regard them as abnormal features. A nucleolus from a germinal vesicle of an unstained ovarian egg is shown in photo. 35. The ovary was fixed in chromo-acetic, washed in water and immersed for one hour in osmic in order to blacken the dentoplasmic granules.

¹ In these vesicles, at a little later stage of development, one of us differentiated in color the nucleolus from the peripheral chromatin ring. Foot, "The Origin of the Cleavage Centrosomes," *Journ. of Morph.*, vol. xii, No. 3, 1897.

After photographing, this ovary was immersed for fifty-three hours in turpentine and the same nucleolus again photographed without staining (photo. 36). The fat granules (osmophile granules) had neither dissolved out nor faded in any part of the ovary. This discredits the advice of those authors who recommend placing sections for twenty-four hours in turpentine or xylol to remove the fat granules before staining. In some cases, perhaps as a rule, warm xylol or turpentine will fade the blackening caused by osmic, but it is by no means infallible, and, moreover, the xylol very often does not dissolve the fat substance. Photographs have enabled us to discover its presence after a careful examination under the microscope had convinced us it had dissolved out completely. Again, after long immersion in turpentine, and after staining, the granules are still sharply blackened, and confidence in the certainty of their removal by turpentine leads one to misinterpret them as other than fat granules.

Further details regarding the nucleoli and the ovarian egg will be discussed and illustrated by photographs in a paper now being prepared for press.

Method.

Further experiments with the mechanical mode of focussing described in our last paper¹ have led to the development of a simpler and more rapid method of overcoming one of the practical difficulties encountered by the cytologist in photography.

After abandoning the effort to focus fine details on the ground glass of the camera, or through a transparent portion marked off in the ground glass, we used the method described in detail in the paper just referred to—ascertaining by experiment with a large object, easily focussed on the ground glass (a sharply stained nucleolus, for example), just what difference the pointer on the micrometer screw registered, between the focus through the microscope and the focus on the ground

¹ "Further Notes on the Egg of *Allolobophora foetida*," *Zoölogical Bulletin*, vol. ii, No. 3, 1899.

glass. This difference we found to be $\frac{3}{20}$ of one of the twenty-five divisions marked on face of micrometer screw.¹

In the practical use of this method, the suggestion for increasing its accuracy came through observing the variation in the turn required by the micrometer screw, dependent upon the operator's eyeglasses, whether reading or distance glasses were worn. This suggested that there must be a lens which, when once adapted to the operator's eyes, would give with unfailing accuracy the plane required for the focus on the ground glass. A series of spherical lenses, from -1.D. to -5.D., were tested in the following manner:

A number of small microsomes were carefully focussed through the microscope, with projection ocular IV (diaphragm at O), the operator wearing ordinary distance spectacles, or in a case where the sight was normal, no glasses were used. The micrometer screw was then turned to raise the focus the number of points found by former tests, to give the camera focus. By this change of screw the microsomes originally focussed upon were of course completely lost sight of. Leaving the screw at this point, the spherical (minus) lenses were tested, beginning with the lowest number, placing them one at a time on the projection ocular, until one was found which brought the microsomes in sight and gave the desired focus, exact in every detail. In making this test, if the camera focus has not been obtained mechanically (*i.e.*, the difference in the two foci measured by points on micrometer screw) the most accurate way of getting at it is to take a series of photographs, focussing through the minus lenses. Beginning with the lowest number, develop each plate as it is taken, until a negative giving the desired focus demonstrates which lens is needed. The proper lens, when found, can be mounted in ordinary spectacle frames, or simply laid on top of the projection ocular, as the operator finds most convenient.

To insure faithful results it is advisable to make a careful sketch, for reference, of a few of the most minute details, in the section to be photographed. After focussing for a

¹ These figures are given merely to illustrate the method, each microscope requiring a special test.

photograph, let the microscope stand undisturbed for at least ten minutes before pulling down the camera, and never attempt to take a photograph unless the focus has held absolutely true during this interval. After the photograph is taken, raise the camera and again examine the preparation to see that the focus has held while the plate was being exposed. Attention should be given to the working condition of the micrometer screw; it should be tight enough to preclude any chance of slipping after the focus is finally adjusted.

A special test must be made for each magnification, but this need not mean undue experimenting, for two magnifications should be adequate for practical use, the lower for photographing entire sections, and the higher for special details, such as centrosomes. Restricting one's photographs, as far as possible, to one magnification, offers a great advantage for comparison of shrinkages.¹

We feel sure that our photographs in this paper are merely a promise of better results. It has been necessary in the reproduction to sacrifice many minor details to bring out a few important points. Our original prints are partly responsible for this, for in them, to insure a successful reproduction of the salient points in a section, we sacrificed the general cytoplasmic structure. For example, we have experimented with photo. 17, and have made a much stronger impression of the cytoplasm, and at the same time have not obliterated the middle-piece in the aster by over-printing.

¹ We have taken a photograph of a stage micrometer at the two magnifications we use, 660 and 950. A print from this negative gives a scale by which any detail in the photograph can be readily measured.

EXPLANATION OF PLATES.

All the photographs, except Nos. 1, 8, 9, 23, 35, 36, and 37, were taken in the winter of 1899, on the Lumière (France) or Nye (Belgium) plates.

In three or four cases a detail in the original print has been slightly strengthened with a lead-pencil, merely enough to overcome in part what is lost by reproduction.

If any of our readers should wish to compare the reproductions with the solar prints, one or more may be obtained on request.

EXPLANATION OF PLATE XXXV.

PHOTO. 1. An entire egg (oöcyte first order). This photograph was taken by Dr. Charles G. Fuller, of Chicago, in the fall of 1893. He focussed on the fertilization cone, and although the magnification is not great, the photograph appears to us as very satisfactory in showing the size of the cone in relation to the entire egg. Fixative, chromo-acetic. Stain, alum-cochineal. The reproduction of the cone in this figure has not been uniformly successful.

PHOTO. 2. Section (3μ) of oöcyte first order. Fertilization cone cut longitudinally. Head of the sperm within the cone, showing a spiral twist. Cytoplasm typical of chromo-acetic preparations at this stage. The osmophile granules are stained. Fixative, chromo-acetic. Stain, iron-haematoxylin. $\times 660$.

PHOTO. 3. Section (3μ) of oöcyte first order. Fertilization cone cut transversely near its apex. Fixative, corrosive-sublimate. Stain, iron-haematoxylin. $\times 660$.

A comparison of photos. 2 and 3 illustrates the relative shrinkage produced by the two fixatives and the difference in the topography of the cytoplasm.

PHOTO. 4. Vignetted section (3μ) of oöcyte first order. Fertilization cone cut transversely about midway between its base and apex. Fixative, osmic and acetic acid. Stain, iron-haematoxylin. $\times 660$.

PHOTO. 5. Section (3μ) of a pathological oöcyte first order. Fertilization cone cut longitudinally. Fixative, corrosive-sublimate. Stain, iron-haematoxylin. $\times 660$.

PHOTO. 6. Vignetted section (3μ) of a polysperm oöcyte first order. The fertilization cone has almost disappeared, but shows that it contained two spermatozoa. Fixative, picro-formalin. Stain, iron-haematoxylin. $\times 660$.

PHOTO. 7. Vignetted section (3μ) of oöcyte second order, showing a sperm aster, with the middle-piece of the sperm near its center. The head of the sperm appears in the next section as a rod, as shown in photos. 8, 16, 17, 18. Fixative, osmic and acetic. Stain, iron-haematoxylin. $\times 660$.

PHOTO. 8. Section (7μ) of oöcyte second order, showing a part of the sperm head contracted into a rod and an indication of the sperm aster, the section being through the periphery of the aster. This photo. was taken by Dr. Fuller, 1894. Fixative, chromo-acetic. Stain, iron-haematoxylin. $\times$ about 400.

PHOTO. 9. Section (7μ) of oöcyte second order, showing male aster, the center of which is stained so deeply that the middle-piece is completely obliterated. Photographed by Dr. Fuller, 1894. Fixative, chromo-acetic. Stain, iron-haematoxylin. $\times$ about 400.

PHOTO. 10. Vignetted section (3μ) of oöcyte second order, showing a part of the head of the sperm surrounded by sperm granules. Fixative, corrosive-sublimate. Stain, iron-haematoxylin. $\times 950$.

PHOTO. 11. Section (3μ) of oöcyte second order, showing a part of a contracted head of a spermatozoön which appears to have been fixed in the act of constricting off a sperm granule. Fixative, Flemmi's fluid (strong). Stain, iron-haematoxylin. $\times 660$.

EXPLANATION OF PLATE XXXVI.

PHOTO. 12. Section (3μ) of oöcyte second order, showing the head of the spermatozoön, its middle-piece within the aster and a part of the tail. (The missing part of the head is in the next section.) Fixative, corrosive-sublimate. Stain, iron-haematoxylin. $\times 660$.

PHOTO. 13. Vignetted section (3μ) of oöcyte second order, showing part of the head of the spermatozoön, the middle-piece, and (probably) a tiny piece of the proximal end of the tail. Stage of development about the same as that of photo. 12. Fixative, 2% osmic in 70° alcohol. Stain, iron-haematoxylin. $\times 950$.

PHOTO. 14. Vignetted section (3μ) of oöcyte second order. Stage of development about the same as that of photo. 13. Fixative, corrosive-sublimate. Stain, iron-haematoxylin. $\times 950$.

PHOTO. 15. Vignetted section (3μ) of oöcyte second order. Stage of development about the same as that of photo. 14. The section has cut transversely two middle-pieces which are within the aster. (The heads of the two spermatozoa are in adjacent sections.) Fixative, corrosive-sublimate. Stain, iron-haematoxylin. $\times 950$.

PHOTO. 16. Vignetted section (3μ) of oöcyte second order, showing the head of the spermatozoön separated from the middle-piece and contracted into a relatively thick short rod. Within the aster the middle-piece and probably a piece of the tail. Fixative, corrosive-sublimate. Stain, iron-haematoxylin. $\times 950$.

PHOTO. 17. Section (3μ) of oöcyte second order, showing the contracted head of the spermatozoön separated from the middle-piece, which is within the sphere, but over-printing has obliterated it. The hyalin globules larger than those seen in the normal living egg at this stage. Fixative, Hermann's fluid *without* acetic acid. Stain, iron-haematoxylin. $\times 660$.

PHOTO. 18. Vignetted section (3μ) of oöcyte second order. Stage of development about the same as that of photos. 16 and 17. Middle-piece not in the center of the sphere. Fixative, corrosive-sublimate. Stain, iron-haematoxylin. $\times 950$.

PHOTO. 19. Vignetted section (3μ) of oöcyte second order. Middle-piece nearly in the center of the sphere. The contracted head of the sperm is in the adjacent section. Fixative, Flemming's fluid (strong). Stain, iron-haematoxylin. $\times 950$.

PHOTO. 20. Vignetted section (3μ) of oöcyte second order, showing a transverse cut through the middle-piece of the spermatozoön, its position in the sphere eccentric. The head of the spermatozoön is in the next section. Fixative, corrosive-sublimate. Stain, iron-haematoxylin. $\times 950$.

PHOTO. 21. Vignetted section (3μ) of oöcyte second order, showing a little later stage of development than that of 16-19. The middle-piece is beginning to disintegrate. The contracted head of the sperm is seen in the next section. Fixative, Perenyi's fluid. Stain, iron-haematoxylin. $\times 950$.

PHOTO. 22. Vignetted section (3μ) of oöcyte second order at about the same stage of development as that of photo. 21, the middle-piece showing like evidences of disintegration. Fixative, picro-formalin. Stain, iron-haematoxylin. $\times 950$.

EXPLANATION OF PLATE XXXVII.

The photos. of this plate show the early stages of development of the male and female pronuclei, four fixatives being represented.

PHOTO. 23. Shows a transverse view of the second spindle, which has reached the anaphase of development. Photo. taken by Dr. Fuller. Fixative, chromo-acetic. Stain, alum-cochineal. $\times$ about 1000.

PHOTO. 24. Vignetted section (3μ) of mature egg, showing five of the eleven chromosomes which have reached the inner pole of the second spindle, the aster rays of which are still present. Fixative, 2% acetic. Stain, iron-haematoxylin. $\times$ 950.

PHOTO. 25. Section (3μ) of mature egg, showing a little later stage of the chromosomes than that of photo. 24. In this section only two of the eleven chromosomes are seen. Within each tiny vesicle is a minute spherical body which we interpret as the first appearance of a nucleolus. Fixative, picro-acetic. Stain, iron-haematoxylin. $\times$ 660. The reproduction of this photograph is not satisfactory. In the solar print the chromatin rings are nearly as sharp as those shown in photo. 24, and on the inner side of each ring there is a tiny sharp granule.

PHOTOS. 26-32 are vignetted sections (3μ) of mature eggs, showing later stages of development of the vesicles, from three to five vesicles in each section.

PHOTO. 31 shows the second polar body on the periphery.

Fixative, photos. 26 and 28, 2% acetic. $\times$ 660. Fixative, photos. 27, 29, 30, and 32, chromo-acetic. $\times$ 660. Fixative, photo. 31, Flemming's fluid (strong). Stain, iron-haematoxylin. $\times$ 660.

PHOTO. 33. Section (3μ) of mature egg, showing part of the head of the spermatozoön breaking up into vesicles similar to those destined for the female pronucleus, *i.e.*, photos. 24-32. Fixative, picro-acetic. Stain, iron-haematoxylin. $\times$ 660.

PHOTO. 34. Vignetted section (3μ) of mature egg, showing a later stage of development of the male pronucleus than that of photo. 33. Only a part of the pronucleus is present in this section. Fixative, chromo-acetic. Stain, iron-haematoxylin. $\times$ 660.

PHOTO. 35. A nucleolus from a germinal vesicle of an ovarian egg, showing the presence of osmophile granules in an unstained preparation. Fixative, chromo-acetic, followed by osmic acid. $\times$ 950.

PHOTO. 36. The same nucleolus (at a slightly different focus) after the sections had been immersed for fifty-three hours in turpentine. Preparation still unstained. $\times$ 950.

PHOTO. 37. Spermatozoön showing spine, head, middle-piece, and tail. This was taken from a slime tube in which the two cocoons were in process of formation. Spine and middle-piece only are stained.