













AN ATLAS  
OF THE  
FERTILIZATION AND KARYOKINESIS OF THE OVUM

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# AN ATLAS

OF THE

# FERTILIZATION AND KARYOKINESIS OF THE OVUM

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## P R E F A C E.



IT is the object of this work to place before teachers and students of biology a series of figures, photographed directly from nature, to illustrate some of the principal phenomena in the fertilization and early development of the animal ovum. In no branch of biological inquiry has knowledge advanced of late with such rapid strides as in the new science of cytology, which deals with the internal phenomena of cell-life. Within the past two decades this science has brought forward discoveries relating to the fertilization of the egg and the closely related subjects of cell-division and karyokinesis that have called forth, on the part of Weismann and others, some of the most important and suggestive discussions of the post-Darwinian biology. These discoveries must in some measure be dealt with by every modern text-book of morphology or physiology, yet they belong to a region of observation inaccessible to the general reader or student, since it can only be approached by means of a refined histological technique applied to special objects not ordinarily available for practical study or demonstration. A knowledge of the subject must, therefore, as a rule, be acquired from text-books in which drawings are made to take the place of the real object.

But no drawing, however excellent, can convey an accurate mental picture of the real object. It is extremely difficult for even the most skilful draughtsman to represent the exact appearance of protoplasm and of the delicate and complicated apparatus of the cell. The best drawing must necessarily be in some measure schematic and embodies a considerable subjective element of interpretation; it is, moreover, impossible adequately to reproduce it in a black and white text-book figure. The photograph, whatever be its shortcomings (and no photograph can do full justice to nature), at least gives an absolutely unbiassed representation of what appears under the microscope; it contains no subjective element, save that involved in focussing the instrument, and hence conveys a true mental picture. Even a technically perfect photograph, however, is defective in that it sharply reproduces only what is seen at a single level of the focus. In using high powers, moreover, the sharp image at the exact focus is always blurred to some extent by indistinct images of higher and lower levels, and this is the case with even the thinnest sections. Protoplasm is thus made to appear in the photograph more coarsely granular than it does to the eye, the asters are less sharp and brilliant, the apparent size of chromosomes and other minute bodies may be slightly exaggerated, etc. Nevertheless, on the whole, these unavoidable defects of the photograph introduce negative rather than positive errors,—they are faults of omission rather than commission,—and I believe that the photographic plates here presented give, on the whole, a clear and accurate impression of the preparations.

How far, then, do the preparations themselves correspond with the conditions existing in life, and what sources of error may be sought in the methods of preservation? This question is always a difficult one to answer in work of this character, since, as a rule, many of the most important elements of cell-structure are invisible in life, and can only be brought to view by means of suitable fixation, staining, and clearing. In the present case, however, the eggs are so transparent as to show many of the phenomena (though by no means all) in life so that the preparations could be carefully tested by direct comparison with the living object. The result of such tests, which have been very carefully made, shows that the methods employed afford preparations whose general fidelity to nature is beyond question, and I believe, for reasons stated below, that they may be trusted even for the minutest details.

The plates of this atlas are reproduced from photographs of the eggs of the sea-urchin, *Toxopneustes variegatus*, Ag., procured at Beaufort, N.C. The eggs, carefully selected from ripe females, were artificially fertilized in sea-water, and preserved at regular intervals. The eggs of this species have the great advantage of being devoid of pigment and very transparent, so that nuclei, asters, and spindles can be clearly seen and their general history followed in life. After testing many different fixing agents it was found that the best results were obtained by sublimate-acetic (80 parts concentrated aqueous solution of corrosive sublimate and 20 parts glacial acetic acid). When properly used, this reagent causes no change of form and no shrinkage or distortion of the internal structures. The finest details of the nuclear and archoplasmic structures are shown with a clearness and brilliancy which far surpass the results of pure sublimate, Hermann's fluid, chromic acid, chrom-acetic, picro-osmic, picro-sublimate, and even Flemming's fluid, though the last-named reagent (weaker formula) gives good results which were used as a check on the sublimate-acetic.

After fixation the eggs were preserved in alcohol, embedded in paraffine, sectioned in the usual manner, and stained on the slide by Heidenhain's iron-hæmatoxylin. The best results were obtained with sections from 3 to 5  $\mu$  in thickness ( $\frac{1}{5000}$  to  $\frac{1}{5000}$  inch), stained twenty-four hours in the hæmatoxylin and differentiated in 1 per cent solution of iron-alum to a bright but delicate blue. The photographs (with the single exception of No. 29) were taken with Zeiss 2 mm. apochromatic oil-immersion, projection-eyepiece No. 4, at an enlargement of 950 to 1000 diameters, and are reproduced without reduction. Details regarding the photographic technique are given in a following note by Dr. Leaming.

The only successful attempt hitherto made to show the early history of the ovum by means of photography is that of Van Beneden and Neyt\* who published twenty-four photographic reproductions of the eggs of *Ascaris megaloccephala* showing many of the more important facts. Admirable as these figures are in many respects, they are nevertheless very defective in respect both to the completeness of the series and to clearness of detail. Their principal defects are due to the fact that the photographs were taken from entire eggs and not from sections. The blurring of images thus procured is so great as to render the photographs very unsatisfactory; and the author's experience has shown that clear and satisfactory negatives can only be taken from sections, and these must be as thin and as sharply stained as possible.

The set of photographs here published is selected from a set of nearly two hundred negatives. The series is incomplete in some particulars and the figures vary considerably in effectiveness; but as circumstances render it impracticable at present to publish a larger number, the series is put forth as it stands, in the hope that it may serve a useful purpose. In order to render the work more useful for students an introduction has been prepared in which the more important facts are briefly reviewed from a general point of view. A series of explanatory text-figures are also given as a key to the phototypes. These figures are from camera lucida drawings of the object (in many cases the same specimen photographed), and although necessarily slightly schematic, they are in no sense mere diagrams (except in a few cases, as stated in the explanations), but are attempts to represent as closely as possible the actual appearance of the object.

I am glad to express my great obligations to Dr. Leaming, to whose skill the excellence of the photographs is largely due and without whose coöperation the present publication of this atlas would have been impossible. My thanks are also due to Mr. Bierstadt for the especial pains he has taken with the reproduction of the plates. It should be added that the process is a purely mechanical one, which involves no retouching of the plates, and the defects of the original preparations appear in the figures without disguise.

EDMUND B. WILSON,

COLUMBIA COLLEGE, May, 1895.

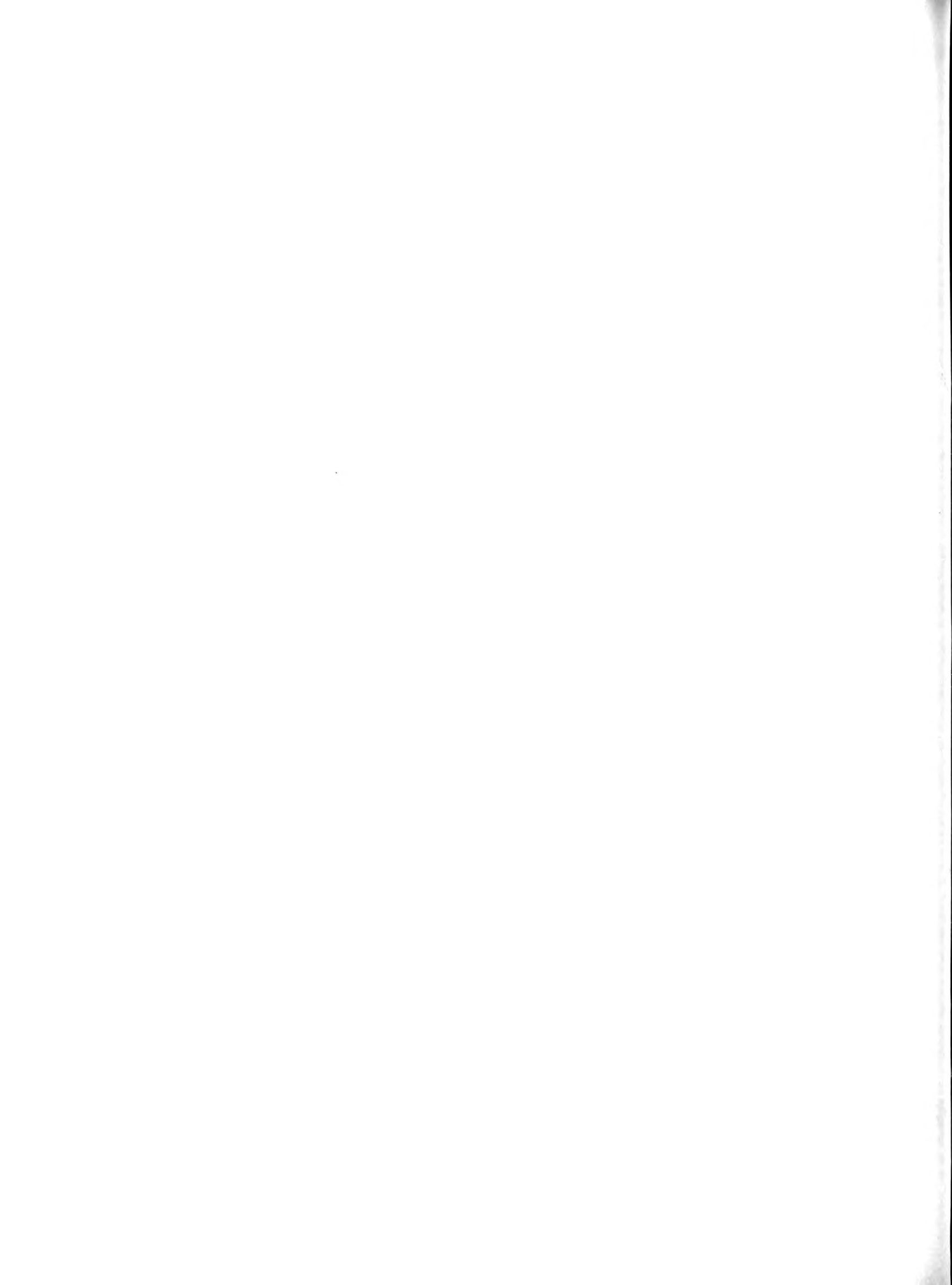
\* Bulletins de l'Académie royale de Belgique, 3 série, June 14, 1887.

## NOTE ON THE PHOTOGRAPHIC TECHNIQUE.

THE installation used in producing the photomicrographs of *Toxopneustes* was that manufactured by Zeiss of Jena in possession of the College of Physicians and Surgeons. In order to have as few variants as possible enter into the work, the following method of procedure and arrangement of apparatus was adopted and adhered to throughout. The adjustment of focus was left entirely with Professor Wilson as being most familiar with the special points desired; the exposure was then made so as to slightly overtime the plate, and it was subsequently intensified; where advisable Strong's adjustable false stage was used in order to bring into the same focal plane a second or third point of interest, and it was found that notwithstanding the short working distance of a 2 mm. lens the slide could be considerably tilted. The optical combination was an Abbe substage condenser achromatic 1 N.A.—a Zeiss 2 mm. oil immersion apochromat and projection ocular No. 4. The camera length was so adjusted that the image represented a magnification of the object of 950 to 1000 diameters. The illuminant employed was the electric arc, which was modified in the following manner. The condenser and objective were so focussed, that the image of the crater was projected with the image of the object on the focussing screen; a finely ground glass screen was then interposed in the path of the light rays near the condenser; this not only gave an evenly lighted field, but prevented the formation of rings or lines by diffraction. The stain used by Professor Wilson being the iron alum and hæmatoxylin stain of Heidenhain, the objects had a light blue tint by transmitted light; therefore isochromatic plates were used with a colour screen made by dyeing a lantern-slide plate from which the silver salts had been removed with an alcoholic solution of tropæolin, a cover glass being cemented to the plate by Canada balsam which rendered the dyed gelatine quite transparent. A single solution hydrokinone developer, of a constant composition, was used, the exposure varying from three minutes with a fresh developer, gradually increasing to ten minutes as the developer became more oxidized. I cannot help alluding here to the excellence of the sections from which these photomicrographs were made, the perfection of which, in every part of the technique of cutting, staining, and mounting, was such as to make working with them a pleasure. In no case have the negatives been retouched or even spotted.

EDWARD LEAMING.

MW, 1895.



## GENERAL INTRODUCTION.

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**The Germ-cells. Ovum and Spermatozoon.**—Since the establishment of the cell-theory by Schleiden and Schwann in 1838-40, the animal ovum has been recognized as being morphologically a single cell, consisting essentially of a mass of protoplasm (cytoplasm) containing a nucleus, and hence morphologically equivalent to any one of the tissue-cells of which the body is composed. The multicellular body is derived from the ovum by a series of successive divisions or cleavages, the egg-cell dividing into two, four, eight, and so on in more or less regular geometrical progression, until a very large number of cells are produced (Text-fig. I. A-L).<sup>1</sup> These cells, known in their earlier stages as *blastomeres*, are ultimately differentiated into the elements of the tissues, and among their descendants a certain number assume the character of the original egg-cell, are converted into ova, and thus form the point of departure for the following generation. Every egg is therefore derived by a continuous and unbroken series of cell-divisions from the egg of the preceding generation, and so on backward throughout all preceding generations; it is normally destined to form the first term in a series of cell-divisions extending indefinitely forward into the future.

In some exceptional cases (*parthenogenesis*), the egg is capable of initiating this series of cell-divisions without the influence of a male element. In sexual reproduction, however, which includes all ordinary cases, both among plants and animals, the egg is incapable of division until it has been fertilized, i.e., acted on by an element derived from the opposite sex and known as the *sperm-cell* or *spermatozoön*. The spermatozoön differs very widely from the ovum in appearance, being extremely minute and provided, in most cases, with a long vibratile tail or flagellum (Text-fig. II. A), by means of which it swims rapidly about. For this latter reason it was long regarded as a parasitic animalcule or infusorian. Not long after the promulgation of the cell-theory, however, it was shown that the spermatozoön, like the ovum, is a single cell, consisting of nucleus and cytoplasm, and that it has a like origin, being derived by division from cells pre-existing in the parent body. Inheritance is therefore effected in both sexes by means of cells, and the mechanism of hereditary transmission is to be sought in cell-structure.

**Fertilization.**—Broadly speaking, fertilization consists in the union of a single spermatozoön with a single ovum, after which the process of division or cleavage immediately begins. It is true that in many cases—for example, in the shark, the butterfly, the earthworm, the newt—two or more spermatozoa may enter the egg. All the evidence goes to show, however, that even in this case, if development be normal, only one spermatozoön plays an active part while the others are passive and sooner or later perish and are absorbed. The fertilized ovum, or oö sperm, is therefore the result of the fusion of two germ-cells derived from the two respective sexes. And since each parent contributes a single germ-cell only to the formation of the embryo, it follows that a single cell is capable of carrying with it the potential sum total of hereditary

<sup>1</sup> Text-figures are numbered throughout with Roman numerals to distinguish them from the phototypes.

characteristics, or stirp, of the parent. The study of the internal changes accompanying fertilization therefore leads directly to an inquiry into the mechanism of inheritance, and our study of these changes cannot be too precise or detailed.

The first decisive discovery regarding the internal phenomena of fertilization was made by Oscar Hertwig,<sup>1</sup> in 1875, in the egg of the sea-urchin. Hertwig determined the fact, namely, that the nucleus of the spermatozoön, or "sperm-

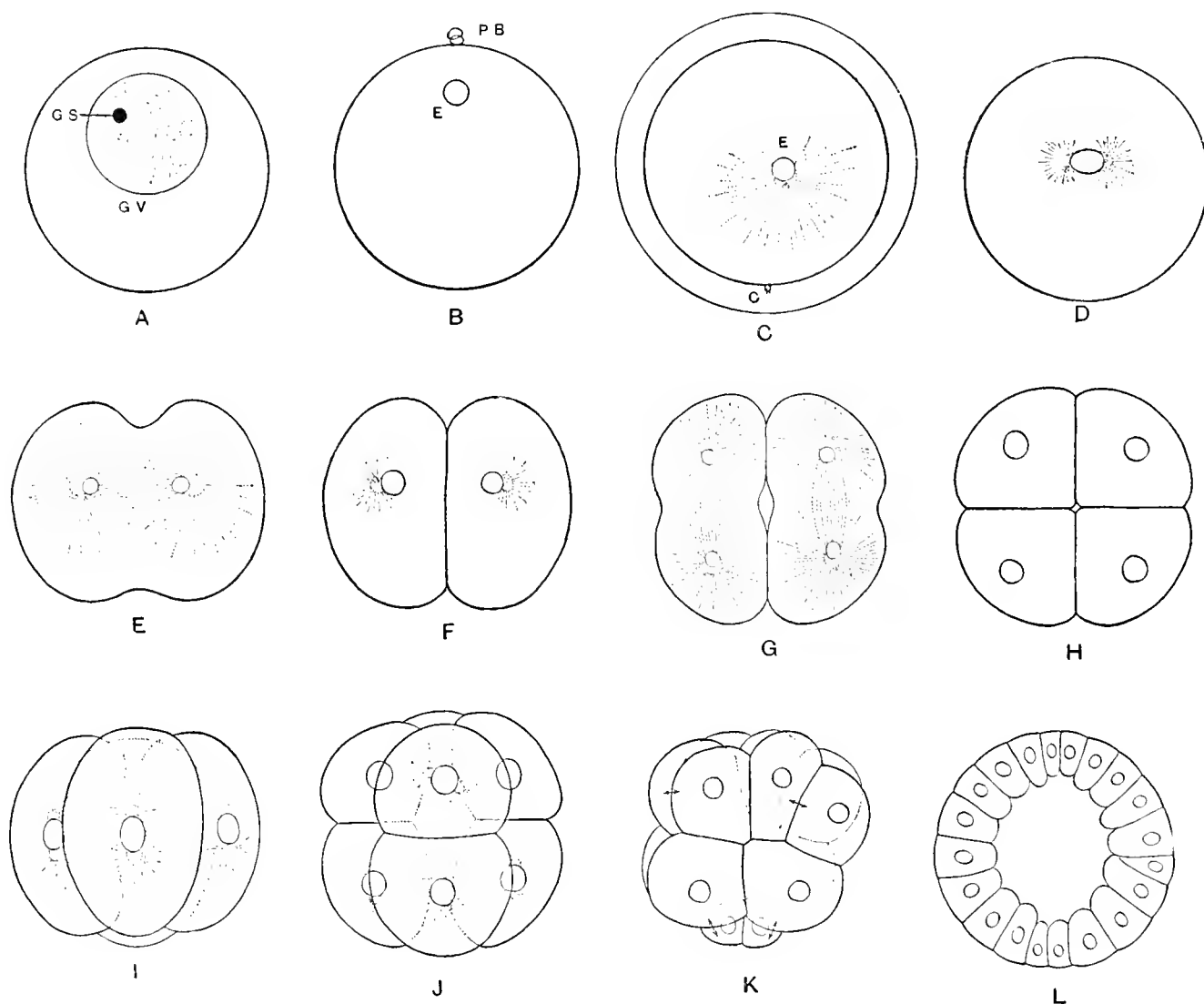


FIG. I.

FIG. I. — Cleavage or segmentation of the ovum of the sea-urchin *Toxopneustes variegatus*. Ag. From the living object. Enlarged 365 diameters.

- A. The unripe ovarian egg, showing the large nucleus or germinal vesicle (GV) containing the nucleolus or germinal spot (GS), and traversed by a delicate network of chromatin.  
 B. Ovarian egg half an hour after the formation of the polar bodies (PB). The egg-nucleus (E) is now much reduced in size.  
 C. Four minutes after entrance of the spermatozoön. Union of the minute sperm-nucleus (shown as a black dot) with the egg-nucleus (E); c. The "entrance-cone" at the point where the spermatozoön has entered.  
 D. "Pause" twenty minutes after entrance, showing the cleavage-nucleus, which has resulted from the union of the egg-nucleus and sperm-nucleus. The sperm-aster has divided to form two asters lying at opposite poles of the nucleus.  
 E. First cleavage, forty-five minutes after fertilization, showing the amphiaster, spindle, and the daughter-nucleus resulting from the division of the cleavage-nucleus.  
 F. Two-cell stage, fifty minutes, showing the daughter-nuclei, each accompanied by an aster.  
 G. Second cleavage, one hour, six minutes.  
 H. Four-cell stage resulting.  
 I. Four-cell stage from the side, showing the asters already divided for the following cleavage.  
 J. Eight-cell embryo, one hour, thirty-eight minutes.  
 K. Sixteen-cell embryo, two hours. At the lower side are four small cells, the "micromeres," that mark the first visible differentiation in the embryo.  
 L. Blastula stage, about three hours. The cells or blastomeres are now arranged so as to surround a central cavity, the blastocel.

nucleus" unites with the "egg-nucleus" to form a single "cleavage-nucleus," which is the parent of all the nuclei of the embryo. This discovery, soon extended to other animals and to the plants as well, gave rise to the view, advocated by Hertwig, Strasburger, Kölliker, Weismann, and many others, that the nuclear substance, or *chromatin* (so named by Flemming), is the most essential element in the germ-cell and must be regarded as the physical basis of inheritance.

<sup>1</sup> Beiträge zur Kenntniss der Bildung, Befruchtung und Theilung des Thierischen Eies. I. Morph. Jahrb., 1875.



**The Chromosomes.**—The analysis was pushed a step farther in the year 1883, by Edouard van Beneden,<sup>1</sup> whose discoveries, originally made in the case of the thread-worm *Ascaris*, were confirmed and extended to many other animals and to plants by Boveri, Strasburger, and others. These discoveries related to the internal structure of the nuclei themselves. In all cases the sperm-nucleus is at first very much smaller than the egg-nucleus, so that at first sight a marked inequality seems to exist between the two sexes in this respect (cf. Text-figs. XI., XII.). Van Beneden, however, determined the remarkable fact that during fertilization the inequality totally disappears and the two nuclei finally exhibit a precise morphological similarity, as follows. Before or during their union, each of them is transformed into a definite number of rod-like bodies, known as *chromosomes* (Waldeyer), *which are of the same form, size, and number in the two sexes*. Both their form and their number differ in different species, but there is strong reason to believe that they are

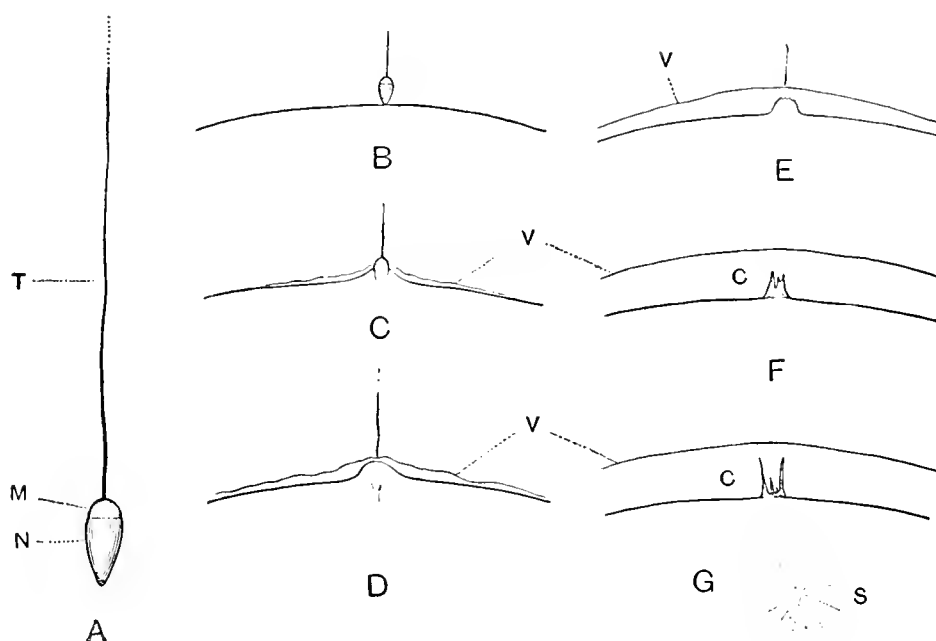


FIG. II.

FIG. II.—The spermatozoon and its entrance into the egg. (*Toxopneustes*. From life.)

A. The spermatozoon, enlarged 3500 diameters. N, nucleus; M, middle-piece; T, tail.

B. First contact with the naked egg (about 1200 diameters).

C. Formation of the entrance-cone and of the vitelline membrane (V).

D. The head (nucleus and middle-piece) has entered the egg. The tail is left outside the vitelline membrane.

E, F, G. Later stages, showing the changes of the entrance-cone. In the last figure (three minutes after the first contact) the sperm-aster has become visible within the egg at S.

always constant in the same species throughout the animal kingdom. Thus, in *Ascaris megalocephala*, each germ-nucleus (egg-nucleus and sperm-nucleus) gives rise in one variety of the worm to one chromosome, in another variety to two (Text-fig. III.). In three other species of thread-worms, *Coronilla*, *Spiroptera*, and *Filaroides*, the numbers are respectively 4, 6, and 8 chromosomes from each sex (Carnoy). The sea-urchin *Echinus*, has 9; the worm *Sagitta*, likewise 9; the medusa *Tiara*, 14; and the mollusk *Pterotrachea*, 16 (Boveri). (Text-fig. IV.) *Toxopneustes* has either 18 or 19 (probably the latter), from each sex. In the trout the number is 12 (Böhm); in the torpedo, approximately 18; and still larger numbers occur in some of the higher animals, few of which, however, have thus far been accurately determined. Precisely parallel results have been obtained among the plants. In the lily each germ-nucleus gives rise to 12 chromosomes (Guignard); in the onion to 8 (Strasburger); in *Pallavicina* (one of the Hepaticæ), the number is only 4.<sup>2</sup>

<sup>1</sup> Recherches sur la maturation de l'oeuf, la fécondation et la division cellulaire. Gand, 1883; also, Arch. de Biol., 1884.

<sup>2</sup> It has recently been shown that the number of chromosomes may vary slightly from the normal, but this is very exceptional. In the snail *Arion*, the sperm-nucleus appears to give rise to only two chromosomes, while the egg-nucleus produces a much larger number (Platner); this exception is, however, probably only apparent.

These facts justify the conclusion that the nuclei of the two germ-cells are in a morphological sense precisely equivalent, and they lend strong support to Hertwig's identification of the nucleus as the bearer of hereditary qualities. The precise equivalence of the chromosomes contributed by the two sexes is a physical correlative of the fact that the two sexes play, on the whole, equal parts in hereditary transmission, and it seems to show that the chromosomal substance, the *chromatin*, is to be regarded as the physical basis of inheritance. Now, chromatin is known to be closely similar to, if not identical with, a substance known as *nuclein* ( $C_{20} H_{10} N_9 P_3 O_{22}$ , according to Miescher), which analysis shows to be

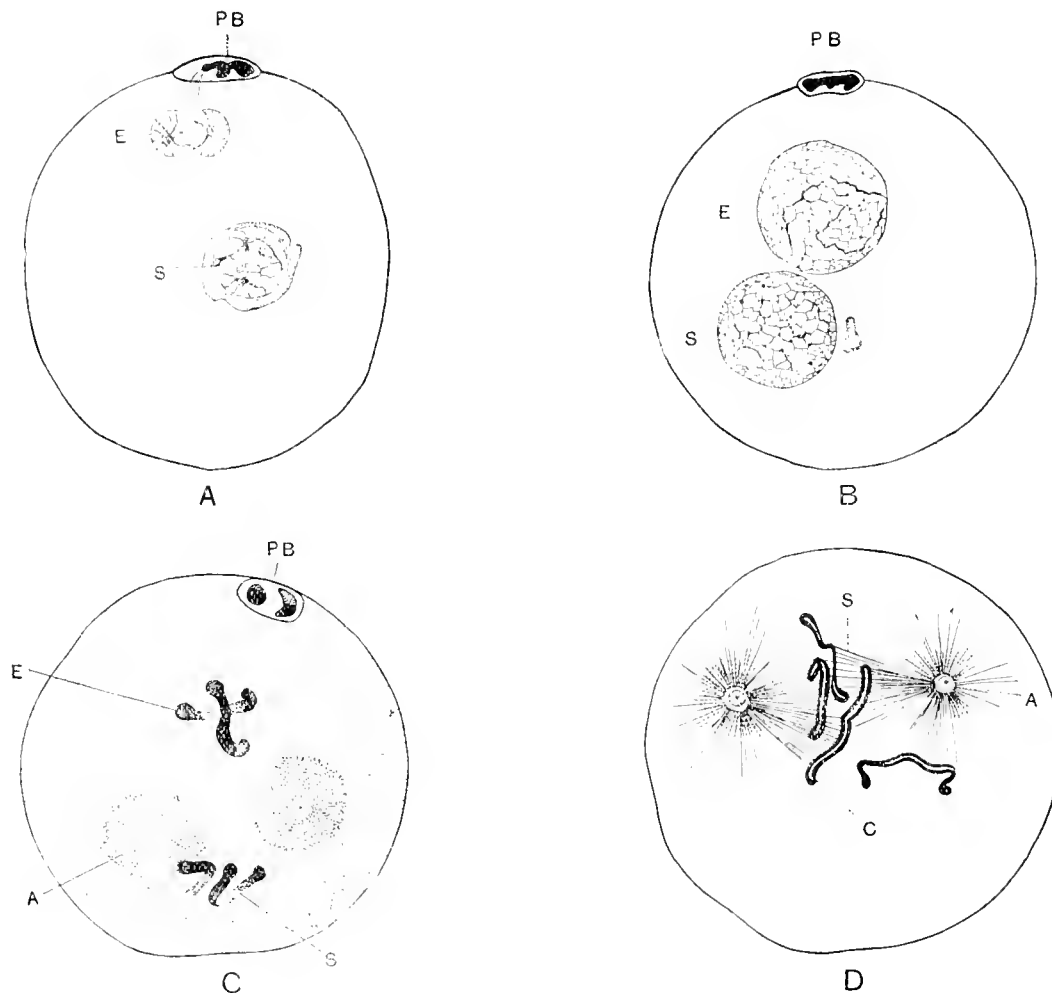


FIG. III.

FIG. III. — History of the germ-nuclei in the thread-worm *Iscaris megalocephalus* (after Boveri), highly magnified.

- A. Egg immediately after the formation of the second polar body, PB; E, the egg-nucleus, consisting of two branching chromosomes; S, the sperm-nucleus derived from the head of a spermatozoön that has entered the egg.
- B. Following stage, in which the egg-nucleus (E) and sperm-nucleus (S) have assumed the same size and structure.
- C. Later stage, in which the substance of each germ-nucleus has become transformed into two chromosomes, — i.e., each sex has contributed two of these bodies, exactly similar in form and size. On either side, at A, is an attraction-sphere or archoplasm-sphere, each containing a single centrosome. These were originally supposed by Van Beneden to be derived one from each sex. Boveri showed that the two arise by the division of one sphere, which is developed under the influence of a centrosome, derived from the spermatozoön alone.
- D. The karyokinetic figure forming for the first cleavage. The archoplasm-spheres have given rise to two asters (A) between which a spindle (S) is forming. The chromosomes (C) are splitting lengthwise, the two halves being destined respectively for the two daughter-cells.

a tolerably definite chemical composed of nucleic acid (a complex organic acid rich in phosphorus) and albumin. And thus we reach the remarkable conclusion that inheritance may, perhaps, be effected by the physical transmission of a particular chemical compound from parent to offspring.

**Structure of the Resting-cell.** — It is now necessary to consider briefly the structure of the resting-cell as an introduction to the subject of cell-division, which, in turn, leads us to another aspect of fertilization.

In the resting-cell (Text-fig. V. A) the protoplasm of the cell-body, or *cytoplasm*, consists of a network or *cyto-reticulum*

the interspaces of which are filled with a mere liquid substance, the *cell-sap* or *cytolymph*. Along the fibres of the reticulum, and especially at its nodal points, are found minute rounded bodies known as *microsomes*. The nucleus is a rounded vesicle, surrounded by a distinct membrane and traversed by a reticulum composed of *linin* fibres, which are probably continuous with those of the cyto-reticulum, and of the same general nature. Suspended in the linin network is a coarser network composed of deeply staining *chromatin*, which is, in many cases, in the form of distinct granules embedded in the linin network. A deeply staining rounded body, the *nucleolus*, is often also present; but this appears to be inconstant.

In many cases, but probably not in all, a portion of the cyto-reticulum is condensed into a definite rounded body lying beside the nucleus and known as the *centrosome* or *centrosphere* (Text-fig. V. A). Its interior sometimes contains one or more minute rounded bodies, known as *centrioles*, but in other cases (as apparently in *Toxopneustes*), there is no centriole, the interior of the sphere consisting merely of a granular or reticular mass.

**Karyokinesis.**—We may now consider briefly the apparatus, by means of which the fertilized egg divides. The usual

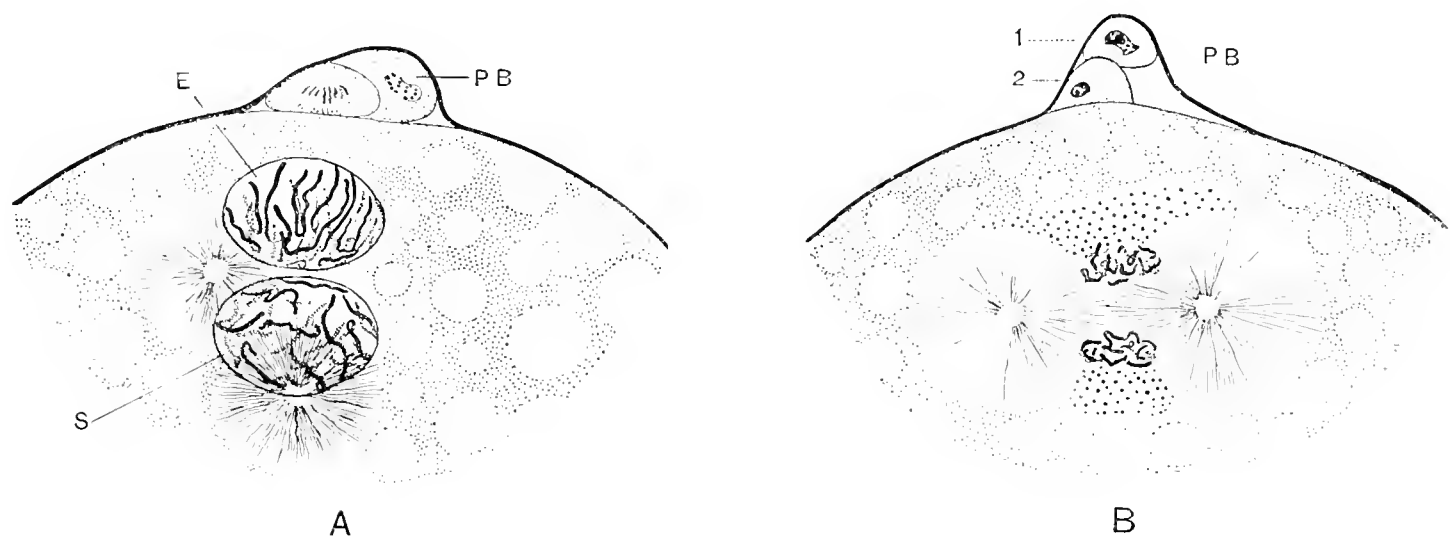


FIG. IV.

FIG. IV. — A. The two germ-nuclei in the egg of the gastropod *Pterotrachea*. Highly magnified, after Boveri. E, the egg-nucleus; S, the sperm-nucleus, each containing sixteen elongated chromosomes. PB, the polar bodies. The centrosome has divided into two to form an amphiasier. Its origin in this animal has not yet been determined. B. Later stage, showing the fully developed amphiasier. Above it lie the sixteen maternal chromosomes, below it the sixteen paternal, the nuclear membranes having disappeared.

form of cell-division (from which the cleavage of the ovum differs only in the fact that it is preceded by a fusion of two cells) involves a complicated process known as *karyokinesis* or *mitosis*. This is characterized by the appearance of a structure known as the *karyokinetic figure* (Text-fig. V. B), derived partly from the nucleus and partly from the surrounding cell-protoplasm or cytoplasm. The karyokinetic figure consists of two elements. One of these, the *achromatic figure* or *amphiasier*, consists of a fibrous spindle-shaped structure or *spindle*, at either pole of which is a star or *aster* consisting of fibres or rays radiating into the cytoplasm, the whole figure strongly suggesting the arrangement of iron filings about the poles of a horseshoe magnet. The centre of each aster is occupied by a rounded mass known as the centrosome or *centrosphere*, which is derived from that of the resting-cell, and like the latter may contain one or more centrioles. The entire substance of the amphiasier is often designated as *archoplasm*, but this term is strictly applied to the substance of the astral rays and spindle-fibres alone. The second, or *chromatic*, portion of the karyokinetic figure is derived from the nucleus, and consists of chromosomes like those arising from the germ-nuclei, which are grouped about the equator of the spindle. As the cell prepares for division each chromosome splits lengthwise into two halves, which diverge to opposite poles of the spindle, and here each group of daughter-chromosomes finally gives rise to

a daughter-nucleus (Text-fig. V. C, D). The cell, meanwhile, divides in a plane passing through the equator of the spindle, so that each daughter-cell contains a daughter-nucleus and one of the asters.

The achromatic figure is undoubtedly to be regarded as a mechanism or apparatus by means of which cell-division is effected, but its precise mode of action is still in doubt. According to the view now generally prevailing (which was originated by Van Beneden<sup>1</sup>), the fibres of the spindle and of the asters are contractile elements, analogous in their mode of action to muscle-fibres. By the contraction of these fibres the daughter-chromosomes are believed to be drawn apart,

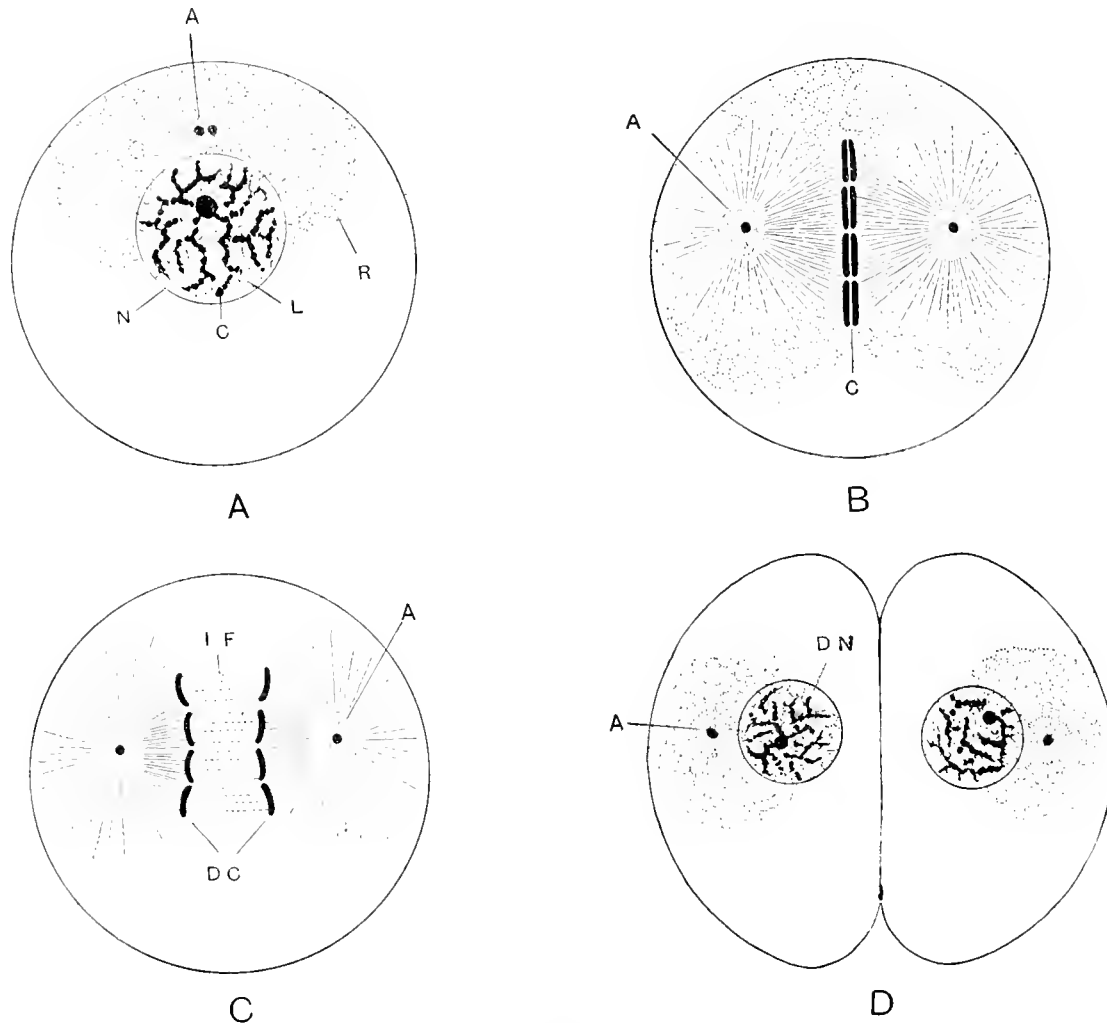


FIG. V.

FIG. V. — Diagrams of the cell at rest and during division.

- A. Resting-cell, showing the cyto-reticulum (R), the linin network (L) within the nucleus (X); C, the chromatin; A, the centrosome containing two centrioles (the latter often regarded as true centrosomes).
- B. Cell in process of division. The centrosome has divided to form two asters (A), each of which contains one of the centrosomes. Between the asters extend the spindle-fibres, which have been formed from the linin network, while the astral rays have been differentiated out of the cyto-reticulum. The nuclear membrane has disappeared. C, the chromosomes (derived from the chromatic network of the nucleus) already split lengthwise.
- C. Later stage of karyokinesis. The daughter-chromosomes (DC) are moving apart towards the respective asters. (IF), the interzonal fibres (Verbindungsfasern) stretching between the diverging chromosome-halves.
- D. The cell has divided into two. Each group of daughter-chromosomes has given rise to a daughter-nucleus (DN), beside which lies a centrosome (A) derived from the preceding aster.

and the cleavage of the entire cell finally effected. Other observers, however (e.g., Strasburger), reject this interpretation and leave the precise nature of the mechanism in doubt. There can, however, be no doubt that the amphiaster is in some manner an expression of the forces by which cell-division is effected.

**Origin of the Amphiaster.** — By the early observers the amphiaster was supposed to disappear entirely at the close of cell-division. In the year 1887, however, the discovery was independently announced by Boveri,<sup>2</sup> Van Beneden,<sup>3</sup> and

<sup>1</sup> Recherches, etc. Arch. Biol., III., pp. 552, 564, etc. See also Van Beneden and Neyt, Nouvelles recherches sur la fécondation, etc.; Bull. Acad. Roy. de Belgique, XIV., 1887, pp. 279, 280.

<sup>2</sup> Boveri. Zellstudien, II., 1888, p. 60.

<sup>3</sup> Van Beneden et Neyt. Nouvelles recherches, etc., I. c., 1887.

Vejdovsky,<sup>1</sup> that the central mass of the aster—i.e., the centrosome—does not disappear at the close of cell-division, but persists as a permanent cell-organ lying beside the nucleus, as already described (Text-fig. V. D). This body forms the starting-point of the amphiaser for the succeeding cell-division, dividing into two halves, each of which becomes surrounded by astral rays, while between them is developed a spindle, formed in part, at least, out of the linin network of the nucleus. The chromatic substance of the nucleus is meanwhile transformed into chromosomes which arrange themselves about the equator of the spindle. This discovery of Van Beneden's, originally made in the case of *Ascaris*, has now been confirmed in a large number of other animals and in plants, and the centrosome has come to be generally regarded as a permanent cell-organ, which presides over the formation of the amphiaser by which the operation of cell-division is effected and the nuclear material is distributed to the daughter-cells.

What now is the origin of the centrosomes in the fertilized egg? Is their substance, like that of the cleavage-nucleus, derived equally from the two sexes? If the centrosome is like the nucleus, a permanent and necessary organ of the cell, then it must be present in each of the germ-cells, as in other cells. On this purely *a priori* ground Rabl asserted, in the year 1889,<sup>2</sup> that the fertilization of the ovum must involve a union not only of nuclei, but also of centrosomes. This prediction was apparently realized in the year 1891, when Fol described in the sea-urchin precisely such a conjugation of centrosomes, as the theory demanded.<sup>3</sup> A similar result was soon afterwards reached by Guignard in the case of the lily,<sup>4</sup> and still later by Conklin, in the marine gastropod, *Crepidula*.<sup>5</sup>

These conclusions regarding the origin of the centrosomes were, however, opposed to those of most earlier observers, all of whom agreed that they were derived solely from the sperm-cell. In *Toxopneustes*, the latter appears to be certainly the case.<sup>6</sup> The amphiaser is in this case developed under the influence of a body contained in the spermatozoön, and located in that portion of the sperm-cell known as the *middle-piece*, which lies behind the nucleus between it and the tail<sup>7</sup> (Text-fig. II. A; see also p. 13). This body is a centrosome, and about it as a centre, after the spermatozoön enters the egg, is developed a single aster, known as the *sperm-aster*, which accompanies the sperm-nucleus in its progress towards the egg-nucleus (Text-figs. IX., X.), and finally divides into two daughter-asters which place themselves at opposite poles of the cleavage-nucleus (Text-figs. XII., XIII.). These daughter-asters become those of the first cleavage-amphiaser, while between them is developed the spindle from the achromatic substance of the nucleus itself. A precisely similar result has been reached in the case of several other animals; in the *Axolotl*, by Fick<sup>8</sup>; in *Ascaris*, by Boveri<sup>9</sup>; in the *Annelid Chætopterus*, by Mead<sup>10</sup>; in the *starfish*, by Mathews<sup>11</sup>; in *Rhynchelmis*, by Vejdovsky<sup>12</sup>; and in several other cases. On the other hand, Wheeler has found in *Myzostomum* that the centrosome is derived from the *egg-cell* alone and not from the sperm-cell.<sup>13</sup>

**Fertilization and Inheritance.**—These facts seem to demonstrate conclusively that the centrosome cannot play any part in hereditary transmission, since it may be derived from one sex only. It is rather to be regarded in accordance with Van Beneden and Boveri as a dynamic element, by means of which is organized the machinery of cell-division. In such cases as *Toxopneustes*, the original centrosome of the egg-cell degenerates and disappears. The egg is, therefore, not capable of cell-division until a new centrosome appears within it. The egg is unable to develop its own centrosome; this organ must therefore be derived from an outside source, namely, the spermatozoön. Fertilization, accordingly, consists

<sup>1</sup> Vejdovsky. See Anatomischer Anzeiger, VI., 1891. p. 370.

<sup>2</sup> Anat. Anzeiger, IV., 1891. p. 29.

<sup>3</sup> Anat. Anzeiger, VI., 1891; also Arch. Sci. Phys. et Nat., XXV., 1891.

<sup>4</sup> Ann. d. Sci. Nat., Bot., 14, 1891.

<sup>5</sup> Wood's Holl Biological Lectures, II., 1893.

<sup>6</sup> See Wilson and Mathews, Journ. Morph., X., 1895. p. 319.

<sup>7</sup> The middle-piece has been traced back in the spermatogenesis to the cen-

trosome of the testis-cell ("spermatocyte"), from which the spermatozoön arises (Hermann, in the *Salamander*; Calkins, in *Lumbriacus*).

<sup>8</sup> Zeit. Wiss. Zool., LVI., 4, 1893.

<sup>9</sup> Zellstudien, II., 1888.

<sup>10</sup> Journ. Morph., X., 1, 1895.

<sup>11</sup> Journ. Morph., X., 1, 1895.

<sup>12</sup> Entwick. Untersuch., I., 1888.

<sup>13</sup> Journ. Morph., X., 1, 1895.

in this case, as in many others, of two distinct phenomena: first, the introduction into the egg of the paternal hereditary characteristics potentially contained in some unknown manner in the substance of the sperm-nucleus or of the chromosomes into which it resolves itself. Second, the introduction into the egg of a centrosome which gives rise to the mechanism by means of which the egg divides and the hereditary substance is distributed to the resulting cells.

Van Beneden and Boveri have shown, in the case of *Ascaris*—and the same is probably true of all other animals and plants—that the substance of the paternal and maternal chromosomes is equally distributed to each of the first two cells (Text-fig. VI.); and there is every reason to believe that this equal distribution of chromatin continues at every

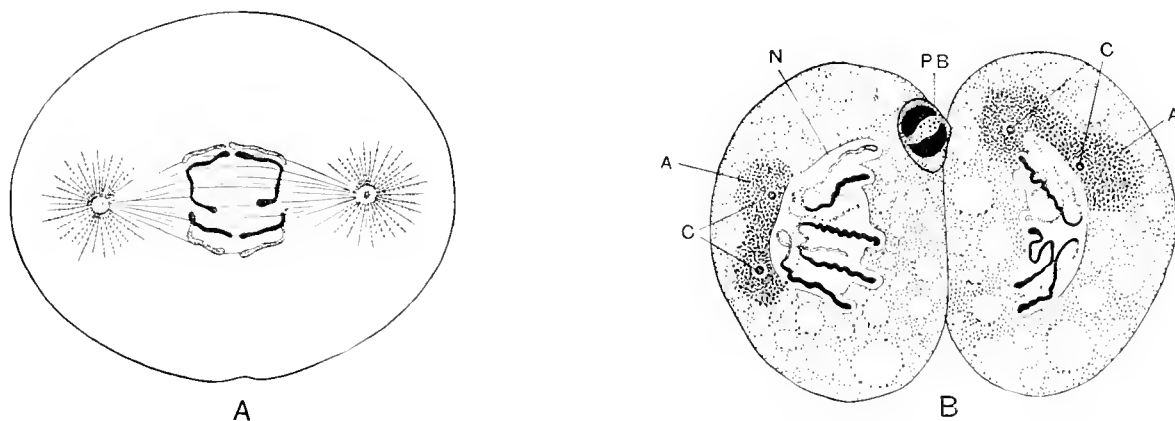


FIG. VI.

FIG. VI. — First division of the egg in *Ascaris* [after Boveri. — Fig. A slightly modified].

A. The chromosomes-halves (cf. Fig. III. D) passing to opposite poles of the spindle.

B. Soon after division, the daughter-nuclei reforming. In each daughter-nucleus are four long chromosomes. Two of these on each side are of maternal origin, two of paternal. Beside each nucleus lies a granular archoplasm-mass (A) derived from the corresponding aster. The centrosome (C) in each has already divided into two preparatory to the next division of the cell. PB, the polar bodies.

succeeding cell-division. In the adult body, therefore, every nucleus of all the myriad cells of which it is composed contains chromatin derived from both the parents. Thus we are enabled to understand, from a physical point of view, that marvellous interweaving of paternal and maternal traits that characterizes the constitution of the offspring.

**Maturation. Formation of the Polar Bodies.** — Before the final union of the germ-cells each of them undergoes a series of changes, collectively known as *maturation*, by which it is prepared for the union with its fellow. These changes affect especially the structure of the nucleus, and in the case of the egg-cell involve the formation of the *polar bodies*, —

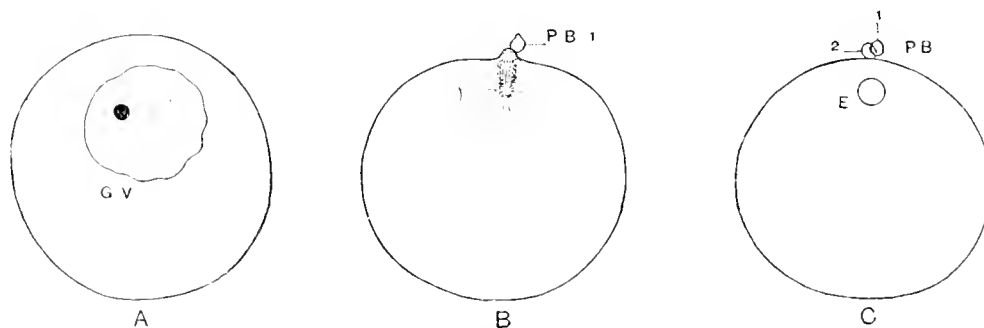


FIG. VII.

FIG. VII. — Formation of the polar bodies in *Toxopneustes*. From life. 365 diameters.

A. Preparatory change of form in the germinal vesicle.

B. The same egg two hours later. The germinal vesicle has disappeared, the first polar body has been extruded, and the second is just forming. The polar amphiastrer is clearly visible, but the chromosomes (dyad groups) are invisible in life.

C. The same egg one hour later. Both polar bodies are still attached to the periphery of the egg. The chromosomes remaining in the egg (i.e., a remnant of the chromatin of the germinal vesicle) have given rise to the egg-nucleus (E).

a phenomenon which occurs before or very soon after the entrance of the spermatozoön. The polar bodies are two minute cells budded forth from the egg, one after another (Text-fig. VII.), which soon perish and disappear without entering into the formation of the embryo. Their precise meaning is not yet fully understood, but their formation is undoubtedly, in some manner, a preparation of the egg-nucleus for union with the sperm-nucleus, since it involves a reduction in the number of chromosomes to one-half the number characteristic of the ordinary tissue-cells of the species.

In *Taxopneustes* and other echinoderms the polar bodies are formed before fertilization, while the eggs are still contained within the ovary; and the same is true of the mammalian ovum, and many others. In many animals, however (e.g., in mollusks and annelids), the polar bodies are not formed until after the spermatozoön has entered the egg. In such cases the sperm-nucleus remains nearly quiescent near the centre of the egg, while the polar bodies are forming (Text-fig. III.).

The formation of the polar bodies is a true process of cell-division, a typical karyokinetic figure being formed which takes up a radial position near the egg-periphery, and results in the division of the egg into two extremely unequal parts. After the formation of the polar bodies the egg-nucleus is reformed as a spherical vesicle, much smaller than the original nucleus (germinal vesicle), and is then ready for union with the sperm-nucleus.

In all cases that have been carefully investigated the number of chromosomes entering into the formation of the polar karyokinetic figure is *one-half the usual number* characteristic of the tissue-cells of the same species. In most cases, but not in all, each of the chromosomes of the first spindle is, from the first, divided into four parts, which form a quadruple group, or tetrad ("Viergruppe," of German authors). In the formation of the first polar body each of the tetrads is halved to form two double-groups, or dyads ("Zweiergruppen"), one of which remains in the egg while the other goes into the polar body. In the formation of the second polar body each dyad is halved to form two single chromosomes, one of which remains in the egg while its sister passes into the polar body (Text-fig. VIII.). It follows, accordingly, that both the

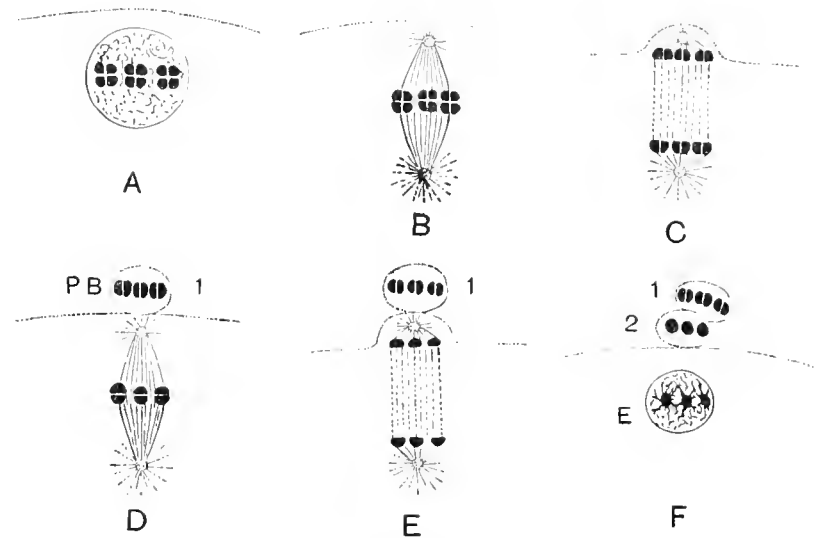


FIG. VIII.

FIG. VIII. — Diagrams to show some of the essential facts in the formation of the polar bodies. The normal number of chromosomes is supposed to be six.

- A. Germinal vesicle containing three tetrad-groups (half the normal number of chromosomes). These have been derived from a portion of the chromatin of the germinal vesicle; the remaining portion degenerates and is absorbed.
- B. The first polar amphiasis with the tetrad-groups (cf. Phototype 3).
- C. Separation of the first polar bodies containing three dyad-groups (each one-half of a tetrad-group).
- D. The first polar body formed. Second polar amphiasis.
- E. Separation of the second polar body with three single chromosomes, leaving three in the egg.
- F. Both polar bodies formed. The egg-nucleus (E) reconstituted out of the three remaining chromosomes (each of them one-fourth of an original tetrad-group). In fertilization the spermatozoön will bring in three chromosomes, restoring the total normal number of six.

egg and the second polar body receive each one-half the number of single chromosomes characteristic of the ordinary somatic cells, while the first polar body receives the same number of dyads or double chromosomes. In some cases the first polar body afterwards divides into two; and it is probable that when this takes place each of the dyads is halved, the two daughter-cells receiving each the same number of single chromosomes as the egg or the second polar body. These facts render it highly probable that the formation of the polar bodies is an expression of the means by which the normal number of chromosomes is reduced to one-half in the egg. A very precise parallel to these phenomena occurs in the formation of the spermatozoön. Here, in like manner, the reduction of the chromosomes to one-half the usual number is effected in the course of the last two divisions before the spermatozoön is finally matured. The mother-cell (spermatogonium) gives rise to quadruple chromosomes of one-half the usual number, which are twice halved in the

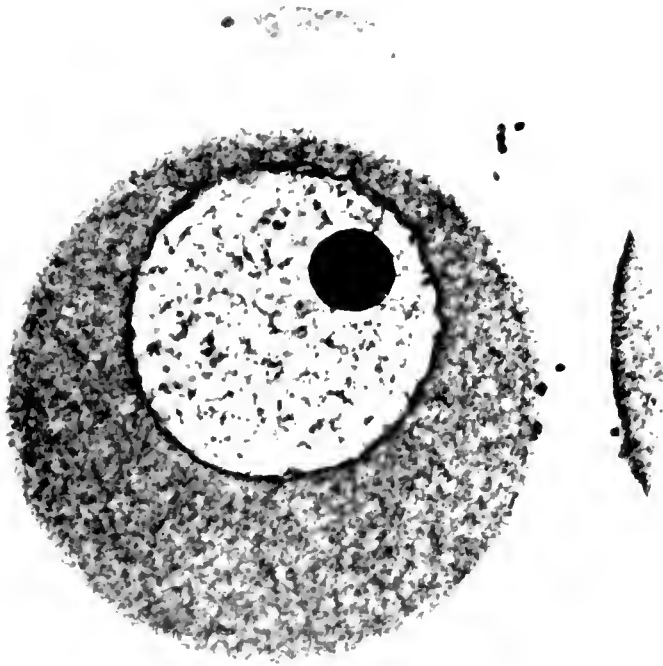
ensuing divisions. From each mother-cell, therefore, arise four spermatozoa, each having half the usual number of single chromosomes. The normal number is restored by union of the two germ-cells in fertilization.

### SUMMARY.

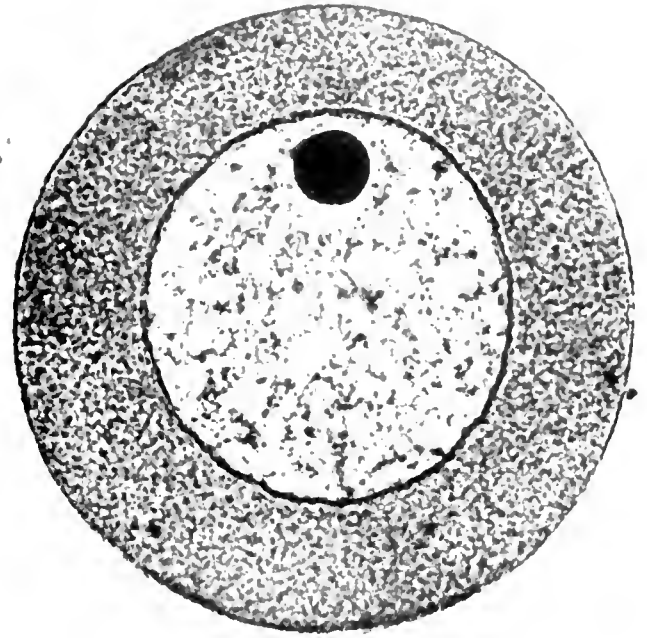
The foregoing facts may be briefly summarized, as follows :

- I. FERTILIZATION. The union of two germ-cells, a spermatozoön and an ovum, derived from the two respective sexes.
  - (a) Entrance of the spermatozoön into the ovum.
  - (b) Union or close association of the two germ nuclei.
 Meanwhile,
  - (1) Transformation of the chromatic substance of each nucleus into a definite number of chromosomes, equal in the two sexes.
  - (2) Origin of a centrosome from the middle-piece of the spermatozoön ; formation about it of a sperm-aster.
  - (3) Fission of the sperm-aster, and development of a spindle between the two halves to form an amphiaster. Grouping of the chromosomes about the equator of the spindle. [Prophases.] The karyokinetic figure formed.
- II. CLEAVAGE. Progressive division or cleavage of the egg. Distribution of the chromatin to the cells of the body.
  - (c) Longitudinal splitting of the chromosomes, and separation of the halves (Metaphase).
  - (d) Divergence of the daughter-chromosomes to opposite poles of the spindle (Anaphases).
  - (e) Reconstruction of two daughter-nuclei from the two groups of daughter-chromosomes, and fission of the entire egg (Telophases).
  - (f) Fission of the aster in each daughter-cell, and formation of a karyokinetic figure in each cell, precisely as before.
  - (g) Repetition of cell-division until a multicellular body is formed. Differentiation of the tissues. Origin of the germ-cells or their immediate predecessors in the reproductive organs.
- III. MATURATION. Reduction of the normal number of chromosomes to one-half.
  - (h) { In case of the ovum. Formation of the polar bodies.
  - { In case of the spermatozoön. The last two cell-divisions in the testis (giving rise to four spermatozoa).
- IV. FERTILIZATION. The cycle completed.

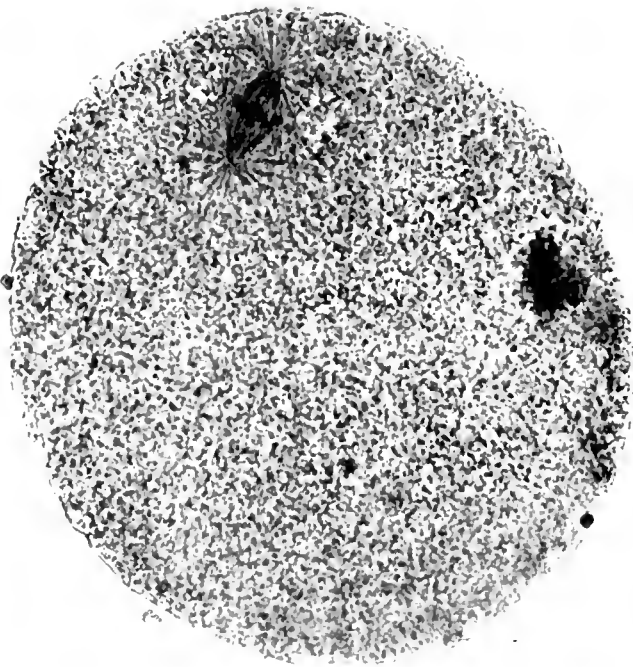




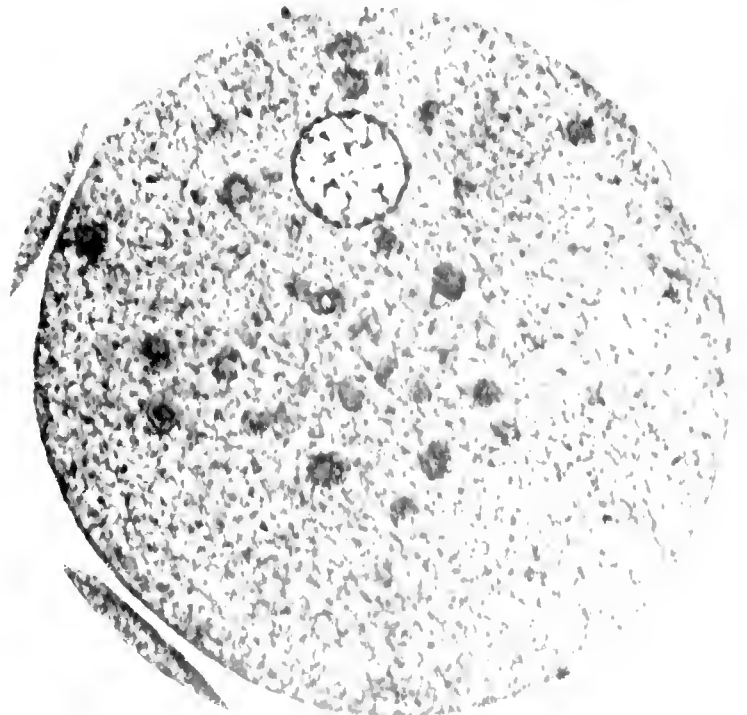
OVARIAN EGG BEFORE FORMATION OF THE POLAR BODIES (P. 11.)



OVARIAN EGG BEFORE FORMATION OF THE POLAR BODIES (P. 11.)



OVARIAN EGG DURING FORMATION OF THE FIRST POLAR BODY (P. 12.)



MATURE EGG, AFTER FORMATION OF THE POLAR BODIES,  
READY FOR FERTILIZATION (P. 13.)



## DESCRIPTIVE PART.



### I. THE UNFERTILIZED EGG. MATURATION.

#### PLATE I. PHOTOTYPE 1.

##### *The Ovarian Egg, before Maturation.*

THE unripe egg, contained in the ovary, is characterized by the enormous size of the nucleus (called in this case the "*germinal vesicle*") and of the nucleolus (or "*germinal spot*") within it (cf. Text-fig. I. A). The protoplasm or *cytoplasm* consists of a delicate network or reticulum (much better shown in some of the later figures) along the threads of which are scattered minute granules, staining deep blue in hæmatoxylin, and generally known as *microsomes*. The *nucleus* or *germinal vesicle* lies always in a somewhat excentric position. It is surrounded by a very distinct membrane (which appears somewhat wrinkled in the photograph), and its interior is traversed by an irregular and discontinuous network of *chromatin*, in which is suspended the *nucleolus* or *germinal spot* (shown as a round black spot in the phototype), stained, like the chromatin, deep blue by the hæmatoxylin.

Close examination of the chromatic network shows that its threads are composed of minute separate granules suspended in a clear achromatic substance, known as *linin*. The clear substance occupying the interspaces of the network is probably a liquid, and is generally designated as the nuclear sap (Kernsaft). The substance of the nucleolus differs widely in its chemical reactions from that of the chromatic network.

The precise relation between the nucleus and the cytoplasm is still a matter of doubt. Most of the recent works on the subject point to the conclusion that the linin network is of the same chemical nature as the cyto-reticulum and is morphologically continuous with it (cf. p. 5). The linin network is, in other words, merely a localized portion of the general cyto-reticulum enclosed within the nuclear membrane. The chromatin-granules (forming the chromatic network) are embedded in the linin and surrounded by it.

#### PLATE I. PHOTOTYPE 2.

##### *The Ovarian Egg before Maturation.*

An ovarian egg that has lain some time in water after extrusion from the ovary. This has caused the germinal vesicle to swell considerably so that its size is somewhat exaggerated, and its excentricity is scarcely apparent. The structure of the ovarian egg appears, however, with diagrammatic clearness.

## PLATE I. PHOTOTYPE 3.

*The first Polar Karyokinetic Figure.*

This figure shows the egg (taken from the ovary) during the formation of the first polar body. An amphiaster has been formed with its axis directed radially toward the surface. The germinal vesicle and germinal spot have disappeared and a large portion of the chromatic substance, including the germinal spot, has been converted into cytoplasm. The remaining portion, which constitutes but a fraction of the original chromatic network, is aggregated to form a group of chromosomes surrounding the equator of the spindle. These chromosomes are arranged in groups of four, — the so-called "*Vierergruppen*," or tetrads, which are characteristic of the formation of the first polar body. (See p. 9.) Their number, which is probably 18 or 19, cannot be made out in the figure. The polar body will be formed at the surface of the ovum (see Fig. VII.), receiving the upper aster with the corresponding spindle-half and half of each tetrad. The second polar body will subsequently be formed at the same point.

## PLATE I. PHOTOTYPE 4.

*The Mature Egg after the Formation of the Polar Bodies.*

The chromosomes remaining in the egg after the formation of the second polar body have become transformed into the egg-nucleus, which appears as a clear vesicle lying in the upper portion of the egg. (The polar bodies, which normally lie at the nearest point of the periphery, have become detached from the egg and are not visible.) The egg-protoplasm (cytoplasm) contains at this period a number of rounded dark bodies stained intensely blue by hæmatoxylin, the meaning of which is not clear. They may perhaps represent the degenerating chromatin not used in the formation of the polar bodies and set free from the germinal vesicle at the time the first polar body is formed. They may perhaps represent yolk-substance (deutoplasm) and form a reserve supply of food. Neither of these views is satisfactory, however, and the real significance of these yolk-bodies remains in doubt.

The egg-nucleus, which is very sharply defined, contains a delicate reticulum of linin (not visible in the figure, since it is not stained by the hæmatoxylin) in the meshes of which are suspended numerous distinct rounded chromatin-granules. The membrane at this period consists of two elements, an outer achromatic layer not visible in the photograph, and an inner chromatic layer composed of chromatin-granules. The inner layer probably is to be regarded as merely the peripheral portion of the general network.

## II. ENTRANCE OF THE SPERMATOZOÖN; ROTATION OF THE SPERM-HEAD; ORIGIN OF THE SPERM-ASTER.

In many animals the spermatozoön enters the egg at a predetermined point, and if the egg is surrounded by a membrane (as, for example, in insects), there may be at that point a special opening or *micropyle* through which the spermatozoön enters. In the sea-urchin the spermatozoön may enter the naked egg at any point. Its lance-shaped head (consisting of nucleus and middle-piece) first comes into contact with the egg periphery by its tip (Text-fig. II. B). Instantly afterwards a rush of the neighbouring egg-protoplasm takes place towards the point of contact, forming a conical prominence, the *entrance-cone*, into which the sperm-head passes. The cone afterwards assumes a ragged flame-shape (Text-fig. II. C, D), and finally disappears a few minutes after the entrance. At the moment the cone is formed a delicate membrane, the *vitelline membrane* (v, Text-fig. II.) is thrown off by the egg. This carries with it the tail of the spermatozoön, attached to its outer surface (Text-fig. II. D, E), so that only the head (nucleus and middle-piece) enters the egg.<sup>1</sup> The vitelline membrane, which entirely surrounds the egg, prevents the entrance of other spermatozoa, and thus avoids polyspermy. The Hertwig brothers have shown that if eggs (of the sea-urchin) be treated with weak solutions of chloral hydrate and other substances, the formation of the vitelline is prevented or delayed, and such eggs are almost invariably polyspermic.

In the living egg the sperm-head usually disappears from view after its entrance, but in its place a small star, the *sperm-aster* (Text-fig. II. G, s), appears two to three minutes afterwards near the entrance-cone. Sections of preserved eggs suitably stained show, however, that the sperm-head persists, and that it rotates within the egg immediately after its entrance, so that the base is turned inwards (Text-fig. IX.). At the same time the middle-piece becomes the centre of a beautiful star-shaped structure, (the sperm-aster) the rays of which arise from the general reticulum. This aster, which afterwards increases enormously in size, is destined to divide into two halves, which, with the spindle developed between them, form the amphiaser of the first cleavage.

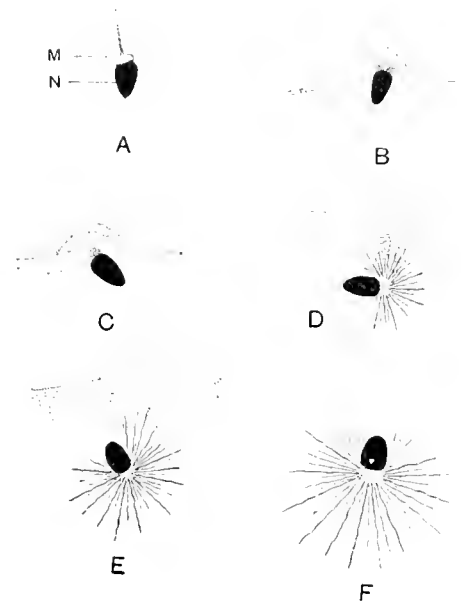


FIG. IX.

FIG. IX.—Entrance and rotation of the sperm-head. Origin of the sperm-aster about the middle-piece. From sections. Enlarged 2000 diameters.

- A. The sperm-head before entrance into the egg, as it appears after double-staining with hematoxylin and Congo red. N, nucleus (deep blue-black); M, middle-piece (pale red).
- B. Immediately after entrance, showing the entrance-cone.
- C. Beginning of the rotation.
- D. Sperm-nucleus rotated  $90^\circ$ ; aster developing about a central mass lying at the base of the nucleus and derived from the middle-piece.
- E, F. Completion of the rotation; growth of the astral rays. The last figure is  $3\frac{1}{2}$  minutes after the first entrance.

### PLATE II. PHOTOTYPE 5.

#### *The First Entrance of the Spermatozoön.*

In this figure the sperm-head may be seen immediately after its entrance as a black, lance-shaped body at the lower pole of the egg opposite to the egg-nucleus. Another sperm-head, which has not entered, is seen at one side, lying at the periphery of the egg. The sperm-head, as thus seen, represents only the nucleus of the spermatozoön. The middle-piece, which lies at the base of the nucleus, is not distinctly visible, since it is stained of the same

<sup>1</sup>In many cases, however, — e. g., in the axolotl, the earthworm, the butterfly. — the tail also enters the egg

colour as the surrounding protoplasm. The tail of the spermatozoön has been left outside of the egg, and is not seen. The entrance-cone is also not seen since it lies slightly out of the plane of section.

PLATE II. PHOTOTYPE 6.

*Inward Progress of the Sperm-head. The Entrance-funnel.*

The egg-nucleus is not shown since it lies out of the plane of section. The sperm-head has advanced somewhat into the egg, leaving behind it a deeply stained funnel-shaped area extending to the surface. In life the entrance-cone (not visible in the section) is seen at the base of this funnel; at its apex is the middle-piece, and from its substance the first rays of the sperm-aster afterwards develop.

PLATE II. PHOTOTYPE 7.

*Unripe Egg abnormally Fertilized. Polyspermy. Entrance-cones.*

The spermatozoa will readily enter unripe eggs which still contain the large germinal vesicle, before the formation of the polar bodies. Such eggs are incapable of forming a vitelline membrane; they are therefore almost invariably polyspermic, as in the present specimen. The entrance-cones are extremely large and persistent, and the section here reproduced clearly shows three of them, while a fourth lies out of focus on the upper side.

In such eggs the spermatozoön never penetrates deeply, never completes its rotation, and never develops an aster.<sup>1</sup> These peculiarities are of great interest as showing how profoundly the egg-constitution is affected by the changes taking place in the nucleus at the time the polar bodies are formed.

PLATE II. PHOTOTYPE 8.

*The Entrance-funnel. Sperm-head Rotated 90°.*

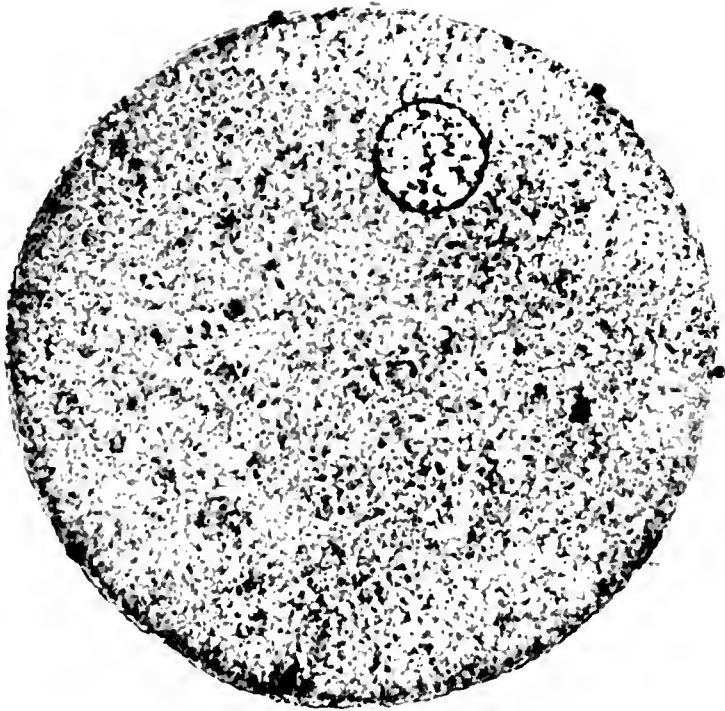
The sperm-head has now advanced still further into the egg, and has rotated through about 90°, its longer axis lying nearly parallel to the surface. From its base the entrance-funnel, now well-developed, extends outwards towards the surface. No astral rays can be seen in this section, but they are clearly visible in the next stage.

PLATE III. PHOTOTYPE 9.

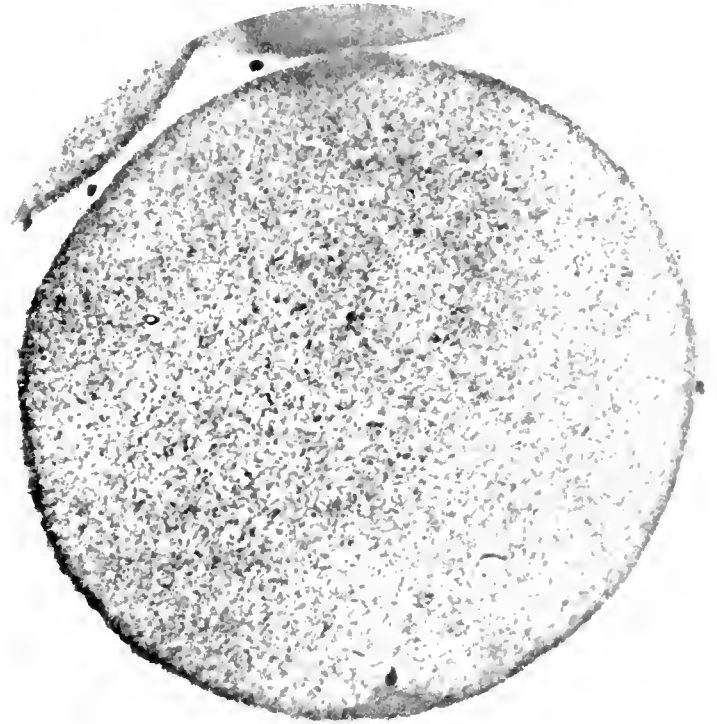
*Origin and Structure of the Sperm-aster.*

A stage immediately following the last in which the astral rays are for the first time visible (cf. Text-fig. IX. D). The sperm-nucleus has rotated rather more than 90°, and is already slightly enlarged, as may be seen by comparison with the sperm-head lying outside the egg at the lower left-hand side. The entrance-funnel is faintly

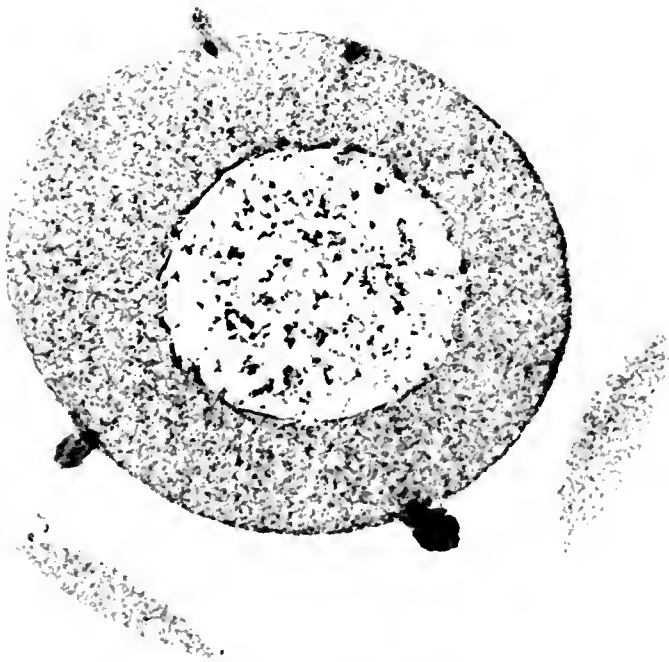
<sup>1</sup> Mathews has, however, observed that a small sperm-aster may be formed in the case of the starfish



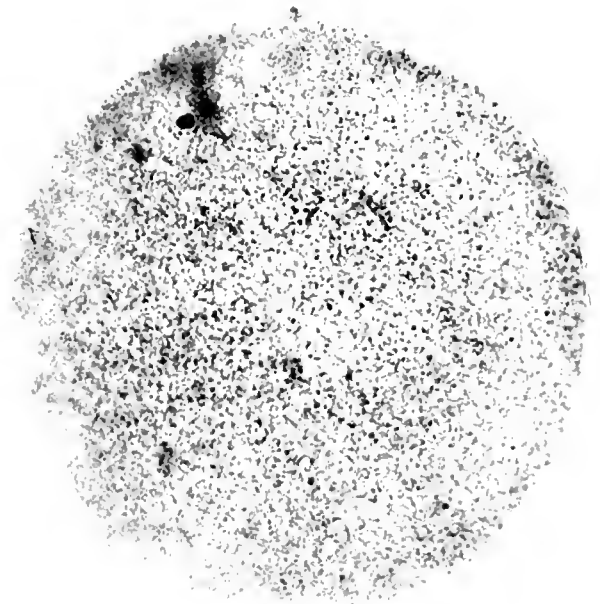
THE EGG IMMEDIATELY AFTER ENTRANCE OF THE SPERMATOZON (P. 14)



SPERM-HEAD ADVANCING INTO THE EGG  
APPEARANCE OF THE ENTRANCE-FUNNEL (P. 14)



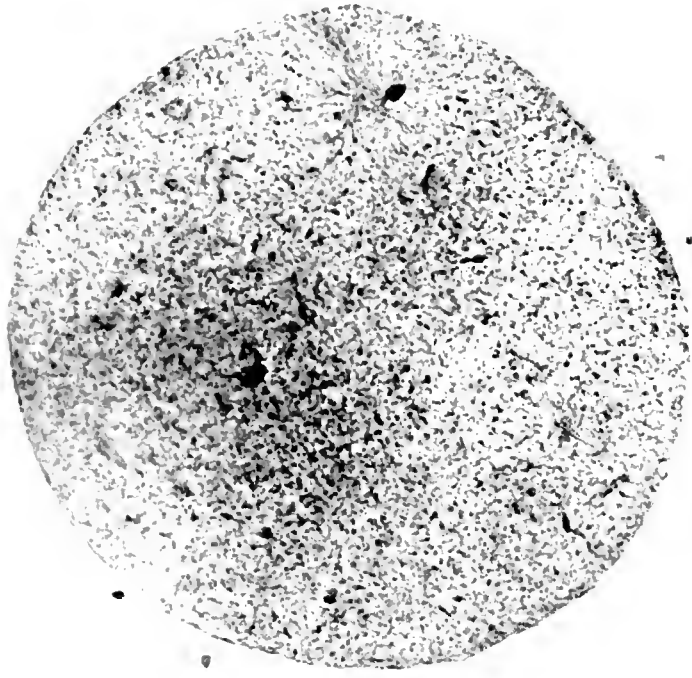
ABNORMAL FERTILIZATION OF UNRIPE EGGS. POLYSPERMIC ENTRANCE CONES (P. 14)



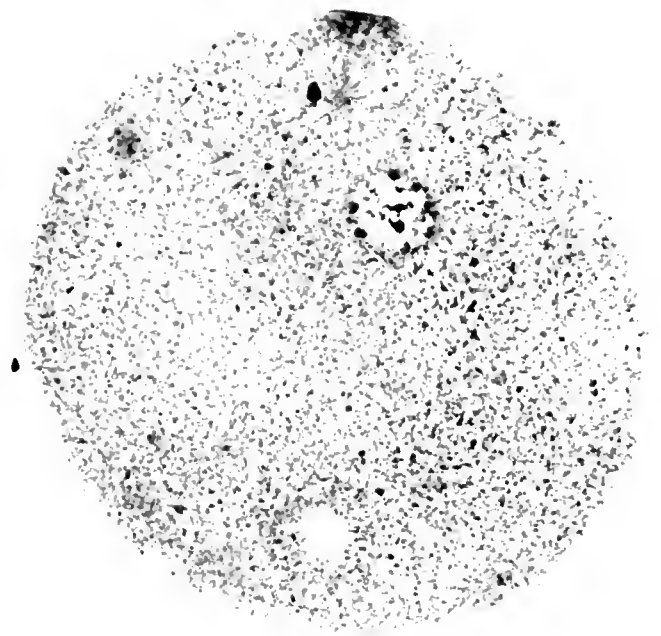
ROTATION OF THE SPERM-HEAD - ENTRANCE-FUNNEL (P. 14)



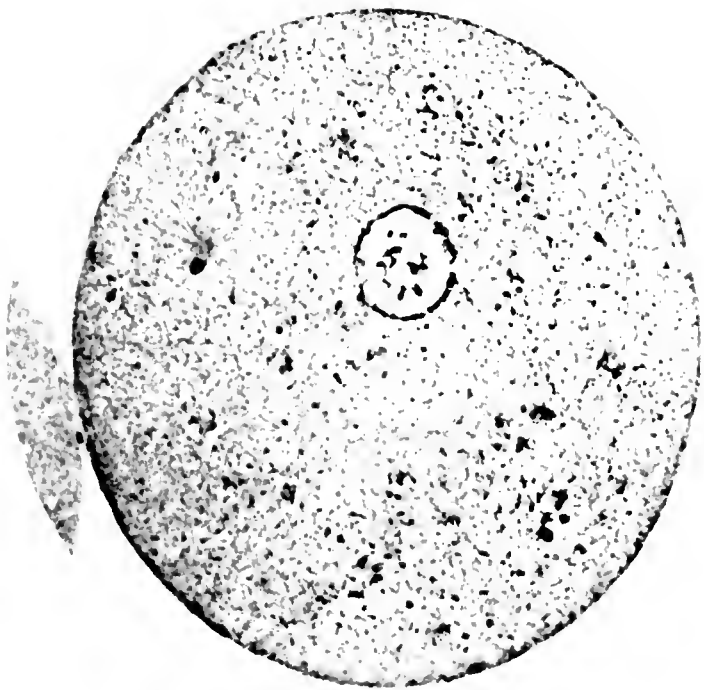




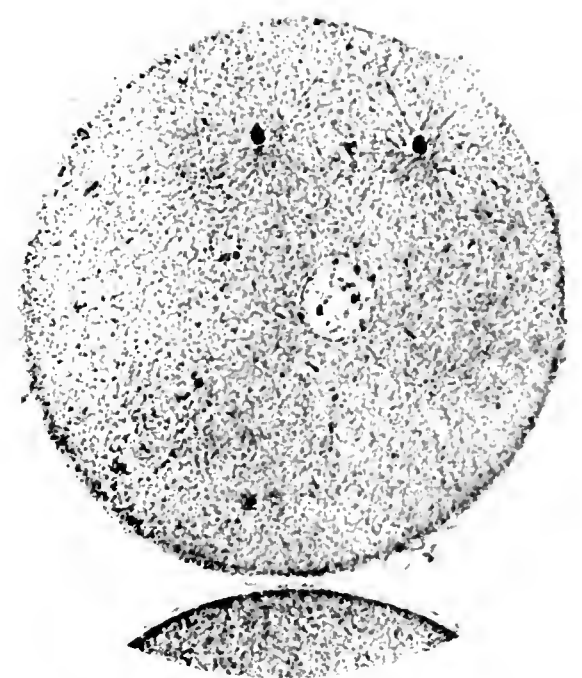
SPERM AFTER DEVELOPING AT THE BASE OF THE SPERM-NUCLEUS (P. 14)



ROTATION OF THE SPERM-NUCLEUS COMPLETED - EARLY SPERM-ASTER (P. 15)



INWARD PROGRESS OF THE SPERM-NUCLEUS TOWARD THE EGG-NUCLEUS - THE SPERM-ASTER ROTATION COMPLETED (P. 16)



DOUBLE-FERTILIZED EGG - WITH TWO SPERM-NUCLEI AND TWO SPERM-ASTERS (P. 17)



shown, extending from the base of the sperm-nucleus outwards to the periphery, where the entrance-cone appears (not quite in focus). The centre of the sperm-aster, which may now be called the centrosphere or centrosome appears as a finely granular mass (see Text-fig. X. A and B), lying at the apex of the funnel and evidently derived from the middle-piece. The rays proceeding from it run out at their tips into rows of granules indistinguishable from the microsomes of the general reticulum, with which they are perfectly continuous. These is reason to believe, as explained beyond, that the astral rays do not properly grow forth from the central mass, as they seem to do, but arise through a radial rearrangement of the pre-existing reticulum under the influence of the centrosome to which they converge.



FIG. X.

FIG. X.— Structure and growth of the sperm-aster; 4000 diameters.

- A. Very early stage (1½ minutes). The middle-piece (now the centrosome) forms a distinct rounded body lying at the base of the sperm-nucleus. A number of short astral rays are forming about it. They may be traced out at their tips directly into the general reticulum.
- B. Later stage (3 minutes). The sperm-head has moved further inwards, its rays are more numerous and extend further out into the cytoplasm. As the figure shows, the rays arise by the direct transformation of the reticulum.

In some cases the central mass of the sperm-aster is described as containing an extremely minute deeply staining body, which has been regarded as representing the true centrosome at this stage (see p. 20). Such a body cannot be seen in *Tropneustes*, and the evidence indicates that the entire central mass (i.e., the substance of the original middle-piece) is here the centrosome.<sup>1</sup>

PLATE III. PHOTOTYPE 10.

*The Rotation Completed. Sperm-aster lying beside the Sperm-nucleus.*

In this somewhat exceptional specimen the sperm-nucleus has completely turned, so that its base is directed inwards, while the aster lies quite at one side. [As a rule the aster remains exactly at the base of the nucleus.] The funnel and entrance-cone are faintly shown, also the egg-nucleus, slightly out of focus. A single lance-shaped sperm-head shown outside the egg.

<sup>1</sup> See Wilson, Archoplasm, Centrosome, and Chromatin in the Sea-urchin Egg. Journ. Morph., Aug., 1895

## III. APPROACH AND CONJUGATION OF THE NUCLEI. GROWTH AND FISSION OF THE ASTER.

After the formation of the aster the two nuclei approach and finally unite with one another, as a rule not far from the centre of the egg. The sperm-nucleus is usually preceded by the aster (Text-fig. XI., Plate IV., Phototype 14), but may lie beside it. Its path is not directly towards the egg-nucleus, but towards a meeting-point which usually lies near, but not at the centre of the egg. The egg-nucleus likewise moves towards the meeting-point, but traverses a shorter path than the sperm-nucleus, since it does not begin to move until the sperm-nucleus has advanced some distance. As a rule the aster meets the egg-nucleus first, and flattens down against it. The two nuclei then come into contact and fuse together to form the *cleavage- or segmentation-nucleus*, and this thereupon moves slowly to its final position (often at some distance from the meeting-point) which is distinctly excentric and never precisely at the centre. Meanwhile the aster divides into two halves (Text-fig. XII. A, B), which place themselves at opposite poles of the cleavage-nucleus (see also Text-fig. I. D).

The movement of the aster through the egg-substance presents some interesting mechanical problems. The structural relations of the astral rays to the cyto-reticulum are such that a bodily movement of translation on the part of the entire aster would seem to be impossible. What seems to take place is a translational movement of the central mass and a concomitant progressive rearrangement of the surrounding reticulum, comparable with the rearrangement of iron-filings in a moving magnetic field.

## PLATE III. PHOTOTYPE 11.

*Inward Progress of the Sperm-nucleus. Delayed Rotation.*

The sperm-nucleus has advanced some distance into the egg, but still lies in a tangential position, the rotation being only about  $90^\circ$ . One of the astral rays is sharply in focus throughout nearly its entire length and may be traced nearly to the egg-periphery. The other rays, though beautifully distinct in the preparation, appear indistinct since they lie out of the focus.

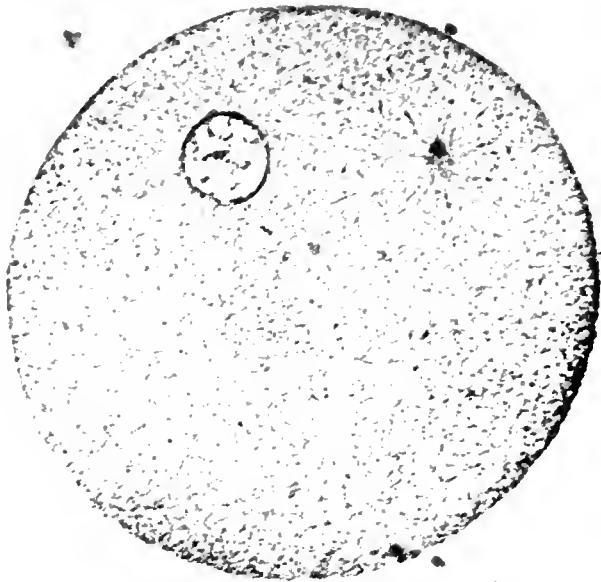
The chromatic portion of the egg-nucleus is here shown with especial clearness, the chromatic membrane (peripheral chromatin) with its pores being very distinct, and also the internal portion of the network. The clear spaces are filled with the nuclear sap traversed by transparent strands of linin clearly visible in the preparation but not shown in the photograph.

## PLATE III. PHOTOTYPE 12.

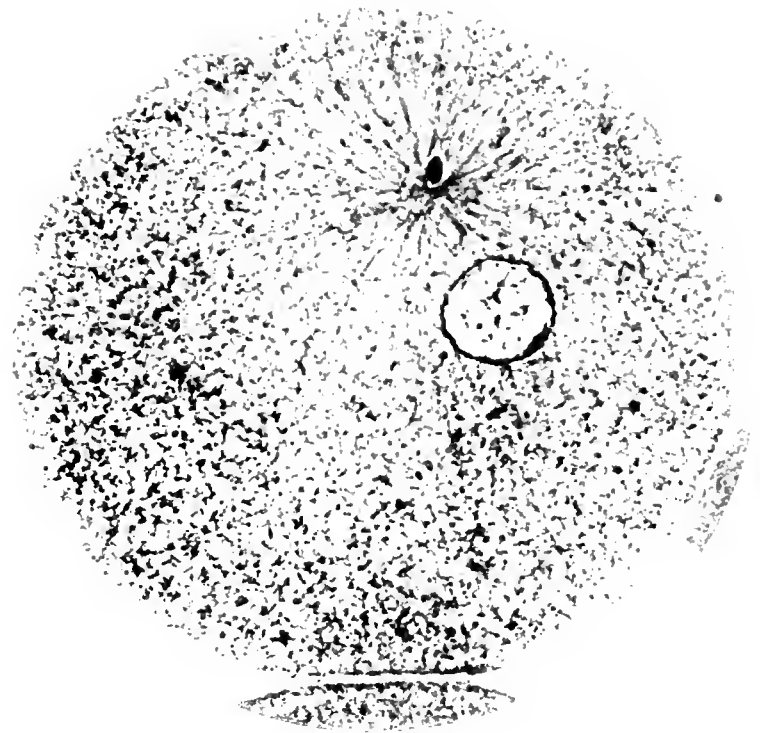
*Double Fertilization or Dispermy.*

This specimen represents a case in which, as often happens, two spermatozoa enter the egg at the same instant. One of the entrance-cones is clearly shown. Each sperm-head has developed an aster, and both are advancing towards the egg-nucleus.

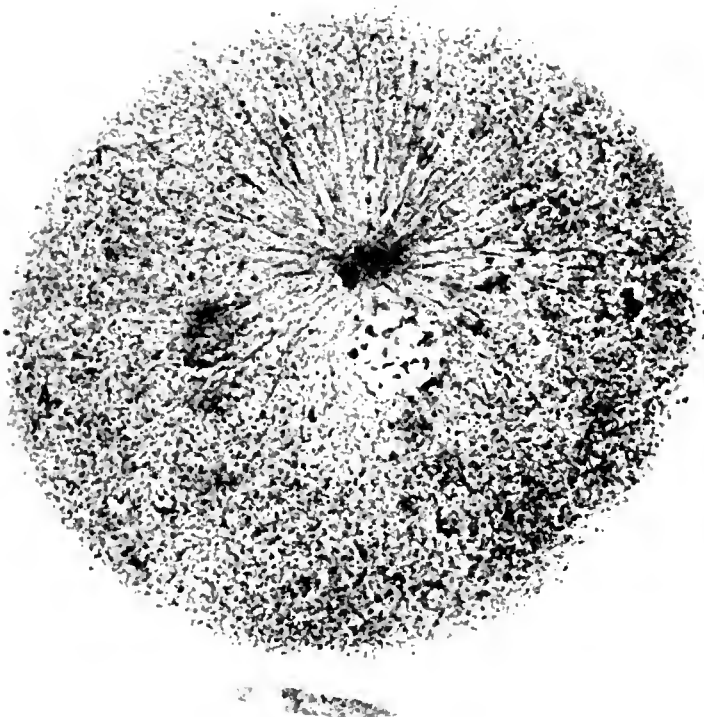
Observations on the living egg show that both sperm-nuclei unite with the egg-nucleus. Each aster afterwards



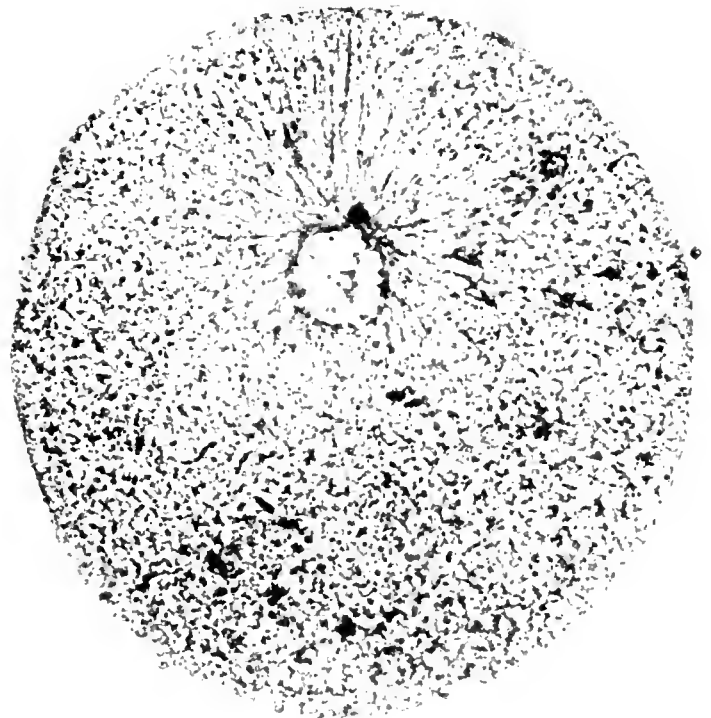
13  
THE SPERM-ASTER ENLARGING (P. 17).



14  
APPROACH OF THE GERM-NUCLEI GROWTH OF THE SPERM-ASTER (P. 17).



15  
THE SPERM-ASTER AND GERM-NUCLEI IMMEDIATELY BEFORE  
UNION OF THE LATTER (P. 17).



16  
FIRST CONTACT OF THE GERM-NUCLEI EIGHT MINUTES AFTER ENTRANCE  
OF THE SPERMATOZOOON (P. 18).



divides into two, thus giving rise to a tetraster or quadruple aster such as is shown in Phototype 22, Plate VI. The eggs thus double-fertilized divide into four (instead of two) at the first cleavage, and the entire cleavage is double, as if the egg consisted of two embryos joined together. They give rise to a blastula stage, apparently normal, but, as Driesch has shown, a gastrula is never produced, and the embryos die without attaining maturity.

## PLATE IV. PHOTOTYPE 13.

*Growth of the Aster.*

A stage slightly later than No. 11 showing the growth of the aster (4 minutes after entrance).

## PLATE IV. PHOTOTYPE 14.

*Approach of the Nuclei.*

Later stage, 5 minutes after entrance, showing the remains of the entrance-cone and the sperm-head completely rotated and approaching the egg-nucleus preceded by the aster (cf. Text-fig. XI. A). The central mass (centrosphere) shows with extreme clearness in the preparation. Its boundary is irregular, its substance finely granular without indication of any centriole. In preparations of this period double stained with hæmatoxylin (blue) and Congo red the astral rays appear blue, while the central mass has a purplish or reddish tone.

## PLATE IV. PHOTOTYPE 15.

*The Sperm-aster at a Maximum (7 minutes).*

The focus is here sharply on some of the astral rays, which are shown with especial clearness, while both the nuclei are slightly out of focus. Many important details of the astral structures are shown. The rays are long, conspicuous fibres, having a wavy course and composed of rows of microsomes which stain bright blue in hæmatoxylin. This is especially apparent in the outer portions of the rays where they run out into rows of granules indistinguishable from those of the general reticulum into which the rays break up. Passing inward toward the centre of the aster, the microsomal structure of the rays becomes less apparent, and they finally fuse indistinguishably to form the central mass, which has an ill-defined contour and a finely granular or nearly homogeneous structure. In sections double stained with iron hæmatoxylin and Congo red or acid fuchsin the astral rays appear intensely blue, while the central mass is red or purple like the original middle-piece, although somewhat obscured by the surrounding blue structure. This contrast between the red central mass and the blue rays becomes extremely conspicuous in later stages, and is described further on. The sperm-nucleus at this period is slightly enlarged, and as a rule has lost its lance-shaped outline.

## PLATE IV. PHOTOTYPE 16.

*First Contact of the Nuclei (8 minutes).*

The central body of the aster has flattened down against the egg-nucleus, and the two nuclei have come together (cf. Text-fig. XI. B). The remains of the entrance-cone faintly appear at the upper periphery. The egg-nucleus has become slightly irregular in contour and shows a number of amœboid processes on the side toward the sperm-nucleus.

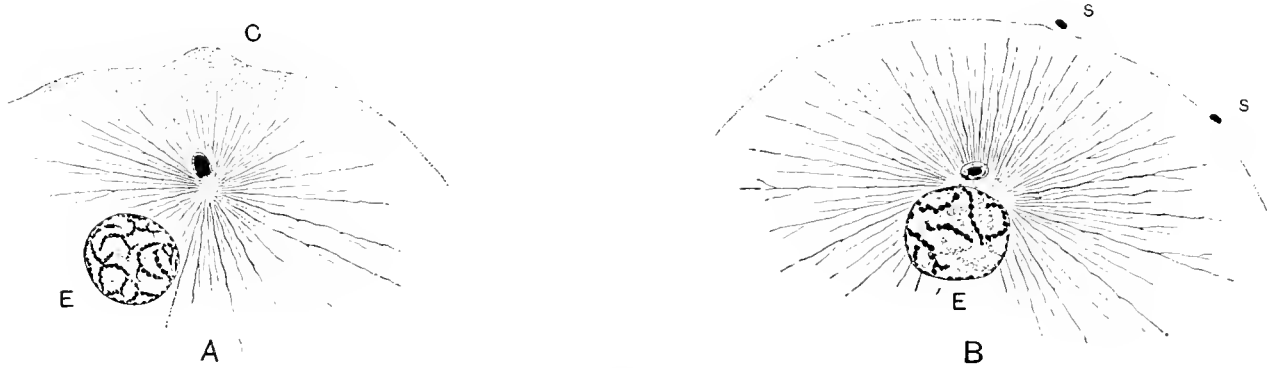


FIG. XI.

FIG. XI. — Approach of the two nuclei (1000 diameters).

- A. Five minutes after entrance. The two nuclei are rapidly approaching (both nuclei move). E, the egg-nucleus. The sperm-nucleus has already enlarged somewhat, and its shape has changed. C, the entrance-cone. (Phototypes 14, 15.)
- B. Seven minutes after entrance. The sperm-aster has greatly increased in size and its central mass has come in contact with the egg-nucleus (E). The sperm-nucleus is now rounded, considerably enlarged, and its substance is less compact. At S, S are two sperm-nuclei lying outside the egg (cf. with that within the egg).

The central body of the sperm-aster lies at a slightly lower level than that at which the photograph is taken, and therefore does not clearly show, but the astral rays are very distinct, passing in all directions toward the periphery of the egg and extending inward more or less around the egg-nucleus.

## PLATE V. PHOTOTYPE 17.

*First Contact of the Nuclei (6 minutes).*

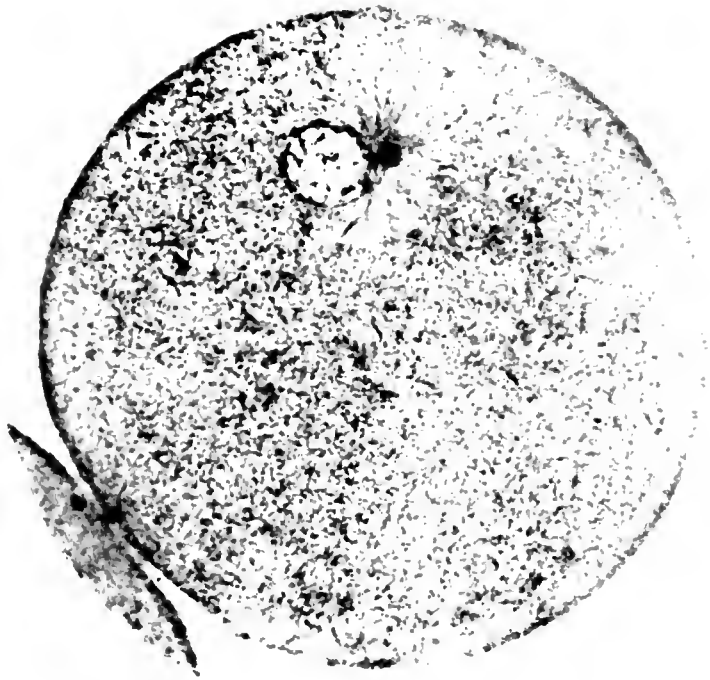
This specimen, like the last, shows the first contact of the two nuclei, as well as the sperm-aster and entrance-cone. It differs, however, in the much smaller size of the aster, which is scarcely larger than in No. 13. This is due (as shown by observation of the living egg) to the fact that the spermatozoön has entered at a point near the excentric egg-nucleus, and has therefore traversed only a short path. The aster has therefore not had time to attain its maximum size.

## PLATE V. PHOTOTYPE 18.

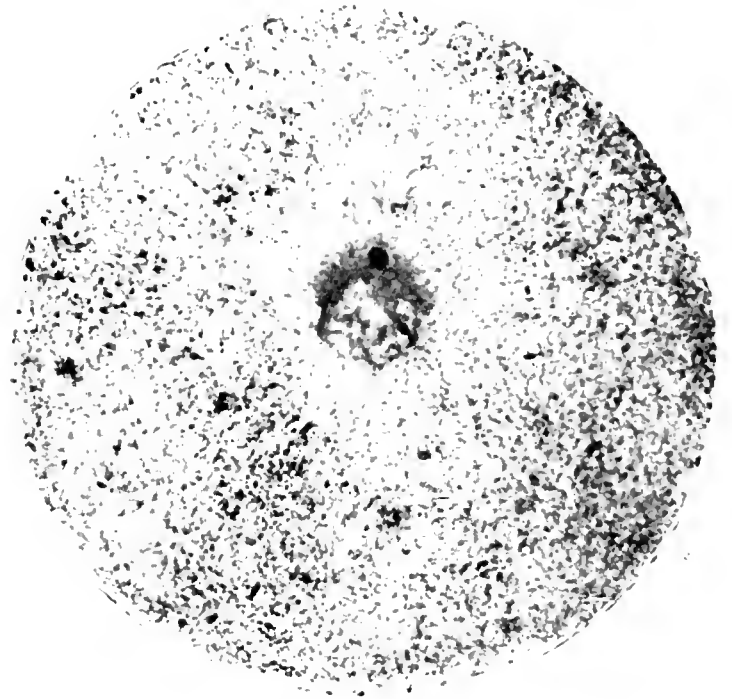
*Extension of the Aster preparatory to its Division (10 minutes).*

This figure is taken from a specimen double-stained with hæmatoxylin and acid fuchsin, and is somewhat indistinct, the astral rays, especially, being scarcely visible. It shows very clearly, however, the central mass of the aster (purple in the section), flattened against the egg-nucleus and extending around it in the shape of a horseshoe. The comparison of

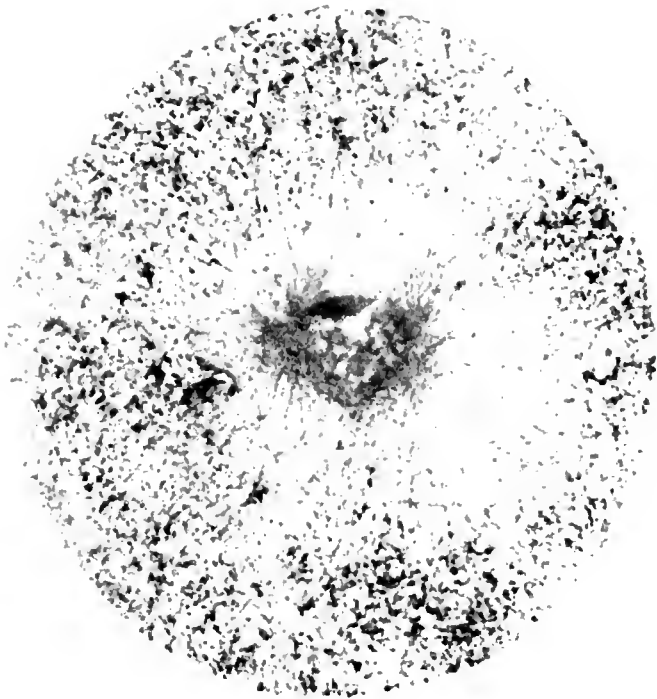




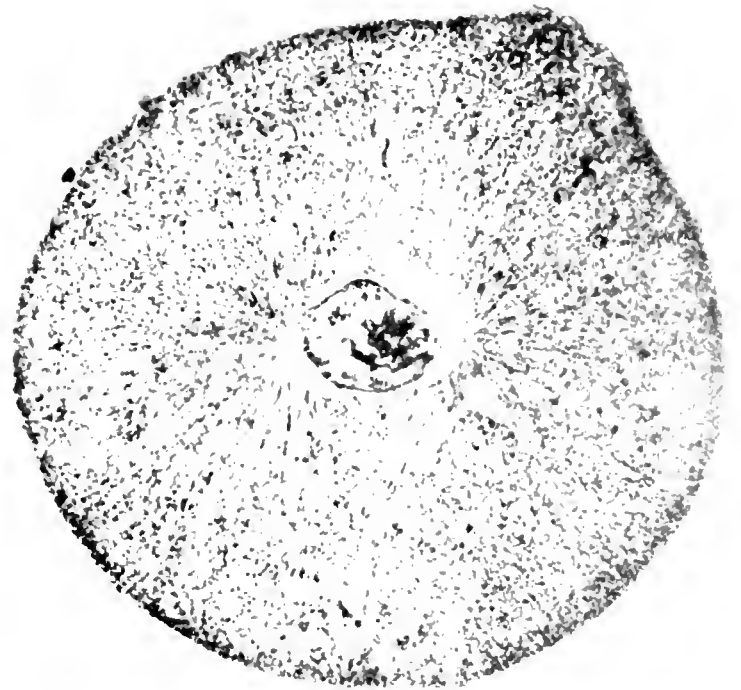
CONTACT OF THE GERM-NUCLEI SIX MINUTES AFTER ENTRANCE. SPERM-ASTER SMALL (P. 18).



THE GERM-NUCLEI IN CONTACT. EXTENSION OF THE ASTER PREPARATORY TO ITS DIVISION (P. 18).



GERM-NUCLEI FUSING. THE ASTER HAS DIVIDED INTO TWO (P. 19).



GERM-NUCLEI FUSING. SPERM-CHROMATID TO THE LEFT. THE RIGHT (P. 19).



sections in various planes shows that the central mass really has the form of a cap, extending downwards in all directions from the sperm-nucleus, which lies at its apex, and is often actually embedded in its substance. The sperm-nucleus is considerably enlarged and its substance is becoming loose and reticulated (Text-figs. XI. B; XII. A).

## PLATE V. PHOTOTYPE 19.

*The Sperm-aster has divided. Nuclei fusing (13 minutes).*

The sperm-nucleus has flattened down against the egg-nucleus (the contour of which is not quite in focus), and appears as a dark lens-shaped body, having a closely reticular structure (cf. Text-fig. XII. B). The sperm-aster has completely divided into two halves which lie at opposite poles of the egg-nucleus. The figure clearly shows the fact that the astral rays from the two sides cross one another nearly at a right angle opposite the equatorial region of the nucleus.

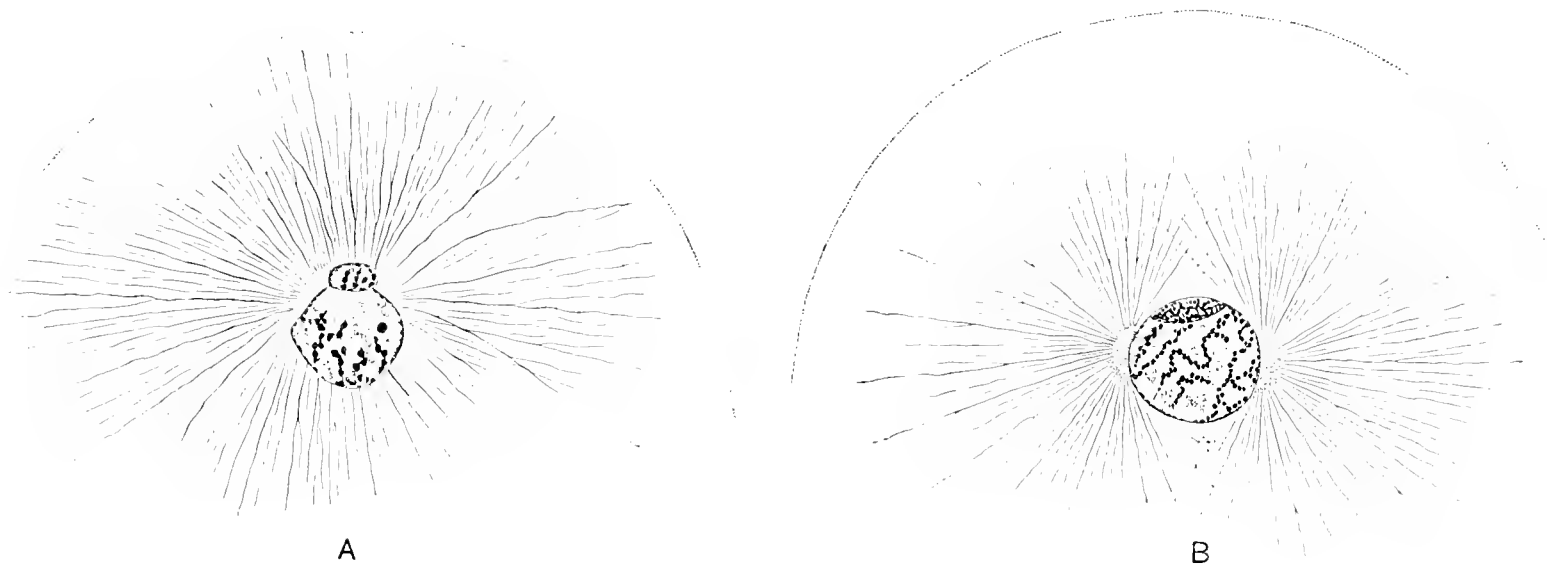


FIG. XII.

FIG. XII. — Conjugation of the nuclei. Extension and division of the sperm-aster (1000 diameters).

A. The two nuclei here lie in contact, the sperm-nucleus considerably enlarged and showing a reticular structure. The sperm-aster has enormously increased; its central mass extends like a cap far down upon the egg-nucleus, and its rays traverse nearly an entire hemisphere of the egg. (Phototype 18.)

B. The sperm-aster has divided into two. The sperm-nucleus is flattened against the egg-nucleus forming a lens-shaped body. (Phototype 19.)

This fact indicates that the rays are really fibres, and not, as some recent authors have maintained, merely the optical sections of thin plates or lamellæ in a radially arranged alveolar structure.

In the European sea-urchin, *Echinus microtuberculatus*, Boveri describes both the germ-nuclei as containing, at this period, nine elongated chromosomes. In *Toxopneustes*, the chromosomes cannot be distinguished until a much later period, and both nuclei consist of an irregular reticulum of chromatin.

## PLATE V. PHOTOTYPE 20.

*Nuclei fusing. Sperm-nucleus en face.*

A stage just later than the last viewed from the side of the sperm-nucleus. The sperm-nucleus appears as a dark irregular mass at the left upper side, its boundary having been nearly lost. The chromatin-granules of the egg-nucleus are distinctly shown.

## IV. THE "PAUSE." FORMATION OF THE CLEAVAGE-NUCLEUS.

Soon after the stages just described, all trace of the distinction between maternal and paternal chromatin is lost to view, and a true *cleavage-nucleus* is formed, containing a uniform reticulum of chromatin. The astral rays, meanwhile, become much shorter and the aster otherwise changes its character. In this condition the egg remains, apparently quiescent, for a considerable period (12–20 minutes), which may conveniently be called the "pause." The most striking phenomenon occurring at this time is the growth of the nucleus, which may increase as much as five or six times in bulk (cf. Phototypes 4, 21, 23, 24, and Text-figs. X.–XIII.). At the same time the aster changes in its staining capacity, the central mass appearing a clear bright red after double-staining with Congo red and hæmatoxylin, while the rays are clear blue.

## PLATE VI. PHOTOTYPE 21.

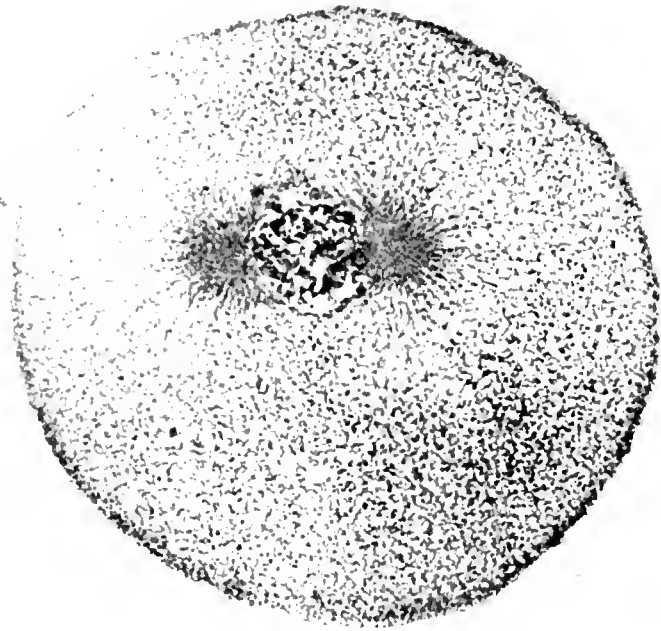
*Typical "Pause." Cleavage-nucleus and Asters (25 minutes).*

This specimen shows especially the asters. The two nuclei have completely fused to form a *cleavage-nucleus* traversed by a close reticulum (much better shown in the following figure). (Cf. Text-fig. XIII. A.)

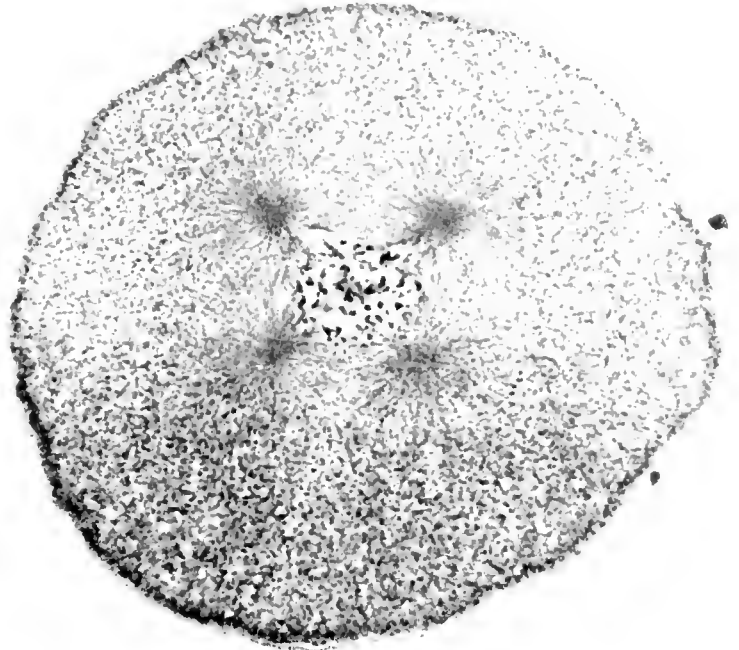
The asters differ strikingly from those of the last stage, the central mass having greatly increased in size, while the rays are very short and their microsomal structure is much more clearly apparent. The central body of the aster ("astrosphere," of Fol; "centrosphere," of Strasburger, "centrosome" of Boveri) is a granular mass staining bright red after hæmatoxylin and Congo red. In some cases (e.g., in the annelid *Chætopterus*, as described by Mead,<sup>1</sup> who has kindly allowed me to examine some of his preparations), the central mass of the aster undoubtedly contains at this period one or two deeply staining centrioles, which in this case may possibly have the morphological value of centrosomes. In *Toxopneustes*, the centre of the aster often contains one or more deeply staining extremely minute granules (smaller than the microsomes, and relatively very much smaller than the centrioles of *Chætopterus*). These bodies doubtless correspond to the "centrosomes" of some authors (e.g., Heidenhain), but in this case they appear to be quite inconstant in number and size and to be merely an early indication of the reticulated centrosphere, afterwards developed within the aster. They afterwards increase in number and are then indistinguishable from the general reticulum<sup>2</sup> traversing the centrosphere.

<sup>1</sup> Journ. Morph., X., 1, 1895.

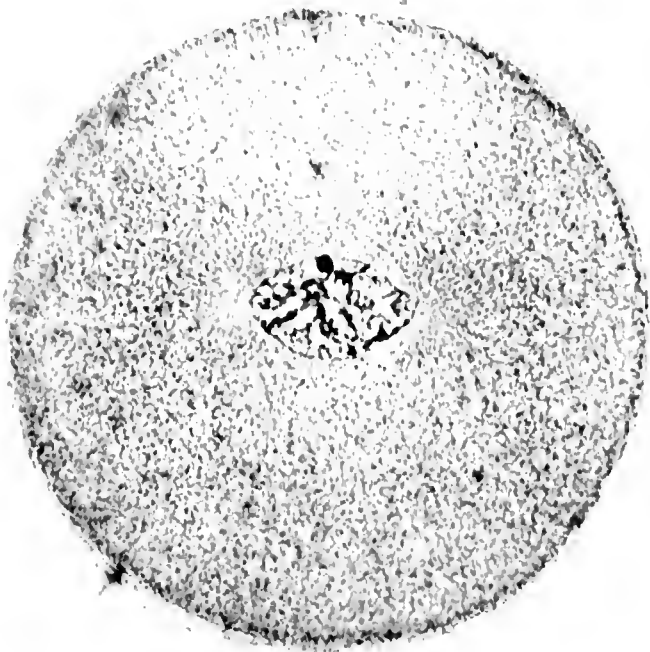
<sup>2</sup> The whole question of the origin and meaning of the "centrosome" is at present in a very unsatisfactory condition. (For a critical discussion see an article by the author in the August number of the Journal of Morphology, 1895, entitled "Archoplasm, Centrosome, and Chromatin in the Sea-urchin Egg.") In Boveri's latest paper (Ueber das Verhalten der Centrosomen bei der Befruchtung des Seeigel-eies, in Verh. d. Phys. Med. Ges. Würzburg, N. F., XXIX., 1, 1895), the word is applied to the entire central mass of the aster, exclusive of the rays—i.e., the "centrosphere" of Strasburger or the "astrosphere" of Fol—and the word "centriole" is suggested for a smaller dark body ("centrosome" of Strasburger) that is often found within it. This terminology is accepted in the present work, though the word "centrosphere" is generally used in place of centrosome, as being less open to misconstruction. The precise relation between centrosome and centriole is still in doubt. It seems certain that the centriole is in many cases a definite morphological body lying within the reticulated centrosphere, capable of growth and division, and showing characteristic staining reactions. But neither can there be any question, in the author's opinion, that the bodies described as "centrosomes" have in some cases been nothing more than artefacts due to a local clotting of the reticulum by the reagents. Such "centrosomes" can easily be produced in the centrospheres of *Toxopneustes* by the use of chromic or picro-osmic acids, and Eismond has recently shown (An. Anz. X. 7, 8, 1894) that the bodies called "centrosomes" (centrioles) in the asters of amphibian blastomeres may have a like origin. It still remains possible that centrioles may exist, even in these cases, but are so minute as to elude detection or are destroyed by the fixing agent, or are not stained, and therefore invisible. There are, however, many grounds for accepting the view that the centriole, though a frequent, is not a necessary, element of the centrosphere. According to Vajdovsky (Ent. Untersuch., I. 1888) the centrosome ("daughter-periplast") is not at first present in the sperm-aster, but arises endogenously within it (i.e. pp. 144, 145); and the centrosome is thus reformed as a centriole at every succeeding division, the original centriole enlarging to become bodily converted into the centrosphere (centrosome of Boveri), while a new centriole appears within it. Sala shows in a recent paper (Arch. Mik. Anat., XLIV., III. 1895) that a perfectly characteristic centrosome may be caused to appear at the pole of the polar spindle in *Ascaris* (in which it is normally represented by a group of granules) by an abnormally low temperature. Heidenhain, who believes that the centrosome (centriole) is an absolutely essential element of the aster (Arch. Mik. Anat., XLIII., p. 651) admits that their number varies, and that they may arise *de novo*,—i.e., not by division or budding from a pre-existing centriole (i.e. pp. 654, 655). Watase considers the centrosome as merely a modified form of the cyto-microsome



21  
PAUSE AFTER FUSION OF THE NUCLEI  
CLEAVAGE-NUCLEUS AND DAUGHTER ASTERS (P. 20).



22  
CLEAVAGE-NUCLEUS AND TETRASTER IN A DOUBLE-FERTILIZED EGG (P. 21).



23  
CLEAVAGE-NUCLEUS DURING THE LATE PAUSE (P. 21).



24  
INITIAL STAGE OF THE KARYOKINETIC FIGURE. CENTRIOLES IN ONE  
OF THE ASTERS (P. 23).



## PLATE VI. PHOTOTYPE 22.

*The "Quadrille." Double-fertilized Egg during the Pause.*

This specimen is the result of a double fertilization such as is shown in No. 12. The two sperm-nuclei have completely fused to form a single large cleavage-nucleus. Each sperm-aster has divided into two, thus giving rise to four asters arranged in a symmetrical tetraster. The egg subsequently divides into four instead of two (see p. 16).

Fol, in 1891, described a "Quadrille of Centres" in the sea-urchin egg (*Strongylocentrotus lividus*) in which two centrosomes derived from the spermatozoön were supposed to conjugate with two corresponding egg-centrosomes. No such process can be seen in *Toxopneustes*, and it is possible that Fol may have been deceived by such specimens as that here shown.

## PLATE VI. PHOTOTYPE 23.

*The "Pause." Showing especially the Cleavage-nucleus.*

The nucleus is elongated preparatory to division. The chromatic reticulum is here very clearly brought out. At the upper margin is a rounded "nucleolus." The asters are now at a minimum, the rays scarcely apparent.

(Journ. Morph., VIII., 1893), and Reinke would distinguish in the cell primary, secondary, and tertiary centres (Arch. Mik. Anat., XLIV., II., p. 276, 1894), which may be formed at any point "je nach dem Bedürfniss der Zelle," the centrosomes being primary centres and formed by the aggregation of tertiary centres or microsomes.

All these considerations, and many others which cannot here be reviewed, indicate, in the author's opinion, that the centriole is essentially an effect and not a cause: that it is a product of a specific form of metabolic action in the "centrosome" of which it forms a part, rather than the inciting cause of such activity.

As regards *Toxopneustes*, the centrospheres show with such beautiful clearness, not only after treatment with sublimate-acetic but also after Flemming's fluid (and in a less degree after pure sublimate), and stain so sharply, that a total destruction of the centriole appears highly improbable. The reticulated centrosphere may be traced back to the group of one, two, or more minute granules visible in the centre of the aster during the early "pause," and a similar group of granules often appears in the centre of the sperm-aster in cases of polyspermy, when the aster remains long undivided. In the normal undivided sperm-aster they cannot, as a rule, be seen, though occasionally present. These granules I believe to correspond to the centrioles of other forms; and the evidence indicates that they are formed endogenously in the aster, and by their growth and multiplication give rise to the reticulated substance of the centrosphere (centrosome, in Boveri's sense). This process is obviously nearly related with that described by Vejdovsky in the case of *Rhynchelmis* (l.c.), though differing from it in the fact that the formation of the daughter-centrioles does not occur until after division of the central mass of the aster as a whole.

## V. THE PROPHASES. ORIGIN OF THE KARYOKINETIC FIGURE.

The origin of the karyokinetic figure (p. 5) involves two processes, viz., 1) the formation of the amphiaser or achromatic figure; and, 2) the formation and division of the chromosomes which form the chromatic figure.

**A. The Amphiaser.** — The asters of the cleavage-amphiasers arise directly from the corresponding halves of the sperm-aster which have persisted through the "pause." The spindle is formed between them, out of the achromatic network (linin) of the nucleus, as shown in Text-fig. XIII., XIV. At the close of the pause (25-35 minutes) the nuclear membrane suddenly fades away at the poles, and in sections the future spindle-fibres may be traced at this point into the interior of the nucleus (Fig. XIII. B), and into continuity with the linin network, now very distinctly differentiated from the chromatin. A few minutes later the nuclear membrane entirely fades away, and a distinct spindle is formed traversing the space between the two asters (XIII. C, XIV.), and completing the amphiaser.

All the evidence goes to show that the spindle-fibres do not properly grow into the nucleus, as they seem to,

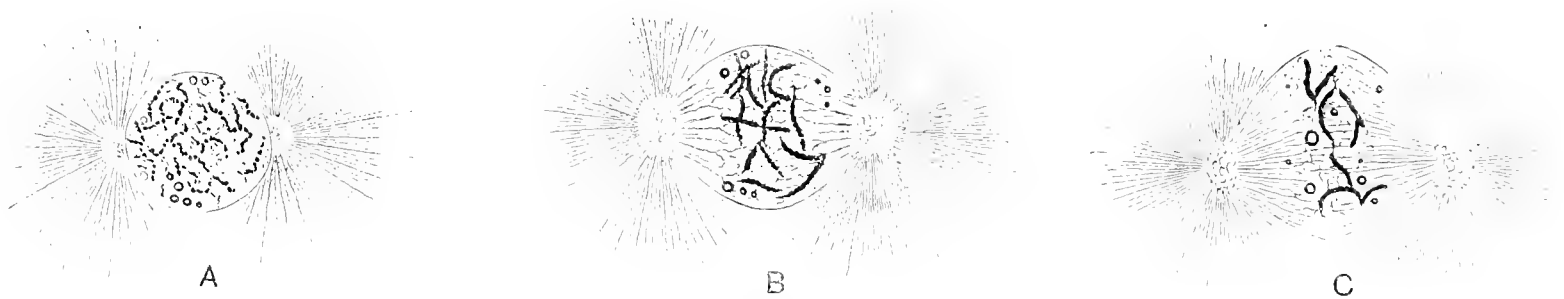


FIG. XIII.

FIG. XIII. — The segmentation- or cleavage-nucleus (1000 diameters).

- A. The "pause" (thirty minutes). The two germ-nuclei have fused indistinguishably to form a cleavage-nucleus, traversed by a chromatin network. Some of the chromatin in the form of rings. The astral rays are much shorter and more granular. In the centre of each aster is a small group of granules.
- B. Initial stage in the formation of the karyokinetic figure. The nuclear membrane has disappeared at the two poles, and the spindle-fibres are forming out of the linin network within the nucleus. The chromatin appears partly in the form of irregular rod-shaped bodies, partly in the form of rings. First indication of the reticulum of the centrosphere.
- C. Slightly later stage, the nuclear membrane fading, the spindle nearly formed. The centrosphere assuming a reticular structure. (Phototypes 21-25.)

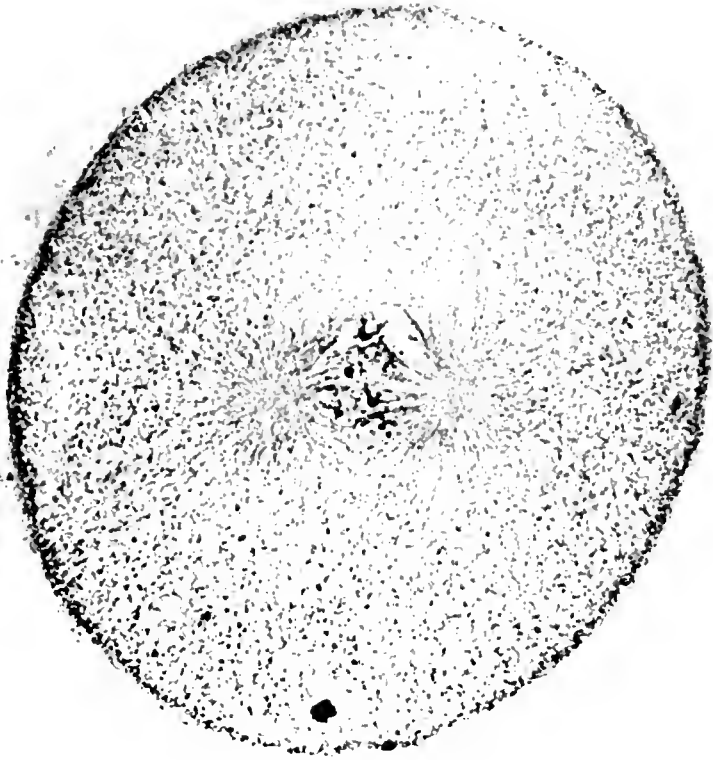
but are progressively differentiated out of the linin network from the aster as a starting-point. This fact explains the contradictory accounts regarding the spindle-formation given by different observers, some of whom describe the spindle-fibres as arising entirely inside the nucleus, others as growing into it from the outside and hence of cytoplasmic origin.

As the amphiaser forms, the centrosphere becomes distinctly reticulated and stains red, so as to contrast very sharply with the blue spindle-fibres and astral rays.

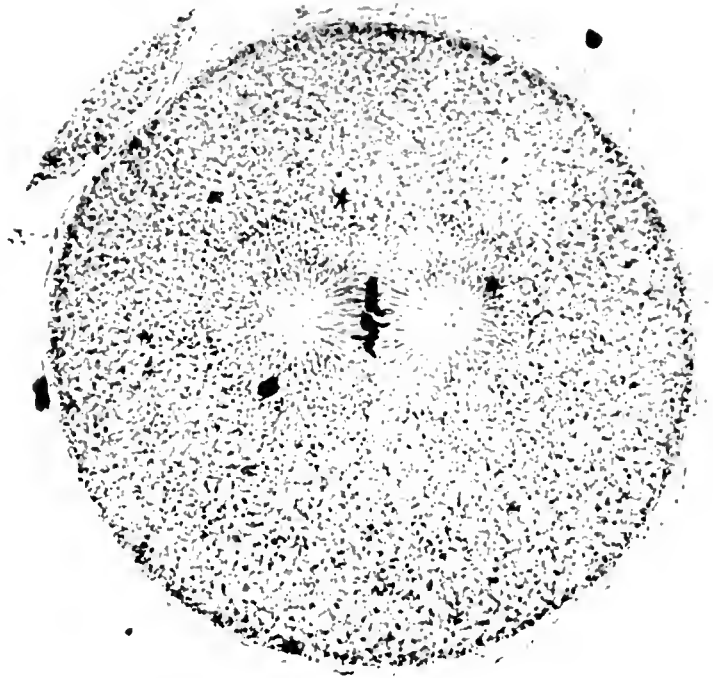
**B. The Chromosomes.** — The chromosomes are always derived from the chromatic reticulum (chromatin) of the nucleus by a morphological rearrangement of its substance, but the *modus operandi* differs considerably in different cases. According to the earlier accounts the reticulum is first converted into a long convoluted thread, forming what is known as the *skein* (Knäuel) or *spirem*. The thread then breaks transversely into rod-like segments which are the *chromosomes*. Later researches demonstrated the fact, however, that the thread is sometimes not continuous but is from the first composed of separate pieces or segments.

The latter is certainly the case in *Toxopneustes*, and no true spirem stage exists. As the nucleus prepares for division, the chromatic reticulum undergoes a remarkable change. A large part of it loses its staining power and becomes indistinguishable from the linin network, which accordingly undergoes a very great and rapid increase in

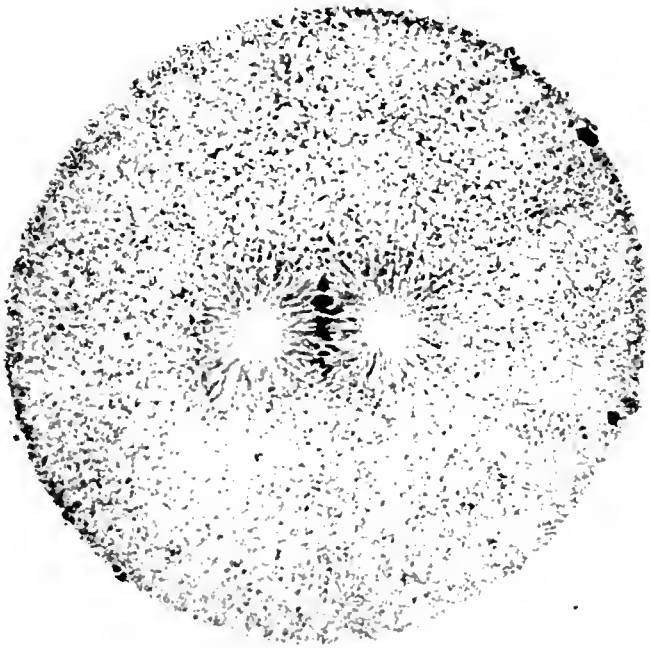




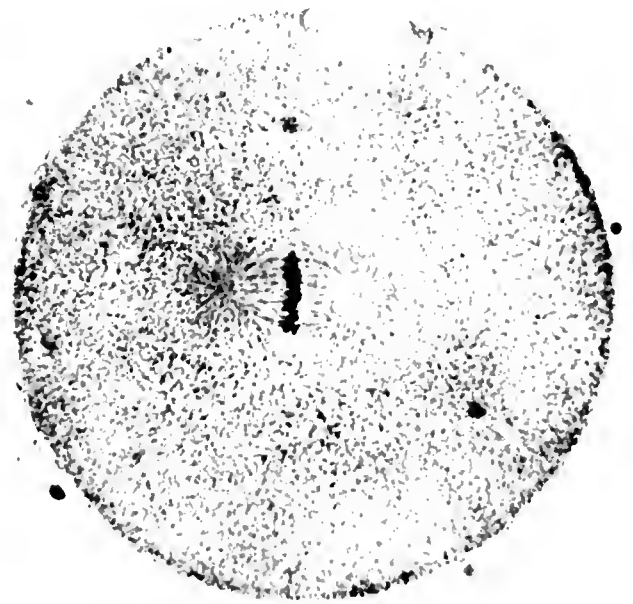
25  
EARLY STAGE OF THE KARYOKINETIC FIGURE.  
NUCLEAR MEMBRANE DISAPPEARING (P. 23).



26  
THE KARYOKINETIC FIGURE ESTABLISHED. CHROMOSOMES  
(EQUATORIAL PLATE) SPINDLE AND ASTERS (P. 24).



27  
EARLY KARYOKINETIC FIGURE. SHOWING THE RETICULAR CENTROSPHERE (P. 24).



28  
EQUATORIAL PLATE DURING THE METAPHASE  
SPLITTING OF THE CHROMOSOMES (P. 25).



amount at this time. From this network the spindle-fibres are formed; hence the evidence indicates that the spindle-fibres are indirectly derived from a portion of the chromatin which is first converted by a chemical change into linin and then, by a morphological rearrangement of the latter, transformed into spindle-fibres. The remaining portion of the chromatin retains its staining power and is finally transformed into the chromosomes, as follows. Most of the residual chromatic network is converted into irregular rods and strings composed of lineally arranged chromatin-granules (Text-fig. XIII. B, C). A part, however, gives rise to hollow spheres or rings of various sizes, which lie scattered about among the chromatin-rods (XIII. A, B, C).

It is very difficult to determine the precise relation of the chromosomes to these two kinds of bodies. Some of the chromosomes appear to arise by the breaking of the rings at one side to form U-shaped rods which finally open out as nearly straight chromosomes; at any rate, every intermediate stage occurs between the closed rings and U-shaped, curved, and straight rods. It is probable, however, that many of the chromosomes (possibly all of them) arise from the longer rods, either by transverse division or by simple shortening.

When fully formed, the chromosomes have the form of rather short rods, at first more or less curved, which are grouped in the equatorial plane of the spindle (Text-fig. XV.). These chromosomes are 38 (possibly 36) in number. It is not possible in this case to distinguish between paternal and maternal chromosomes, since their individuality is entirely lost to view during the pause, owing to the complete fusion of the germ-nuclei. There can be no doubt, however, that one-half the chromosomes of the equatorial plate are derived from each sex as in *Ascaris* or *Pterotrachea* (cf. p. 5).

PLATE VI. PHOTOTYPE 24.

*Origin of the Spindle. Differentiation of the Reticulum.*

The specimen clearly shows the spindle-fibres passing from the aster at each pole into the interior of the nucleus. The microsomal structure of the astral rays is clearly apparent. The small vague dark mass shown in one of the asters is a group of granules (centrioles) like those shown in Text-fig. XIII. A, which form an early stage in the development of the reticular centrosphere (cf. foot-note, p. 20).

The phototype shows, further, the differentiation of the nuclear reticulum into a more deeply staining part, the *chromatin*, arranged in strings and rounded masses, and a less deeply staining portion, the *linin*, into which the spindle-fibres are continued (cf. Text-fig. XIII. B).

PLATE VII. PHOTOTYPE 25.

*Early Stage of the Karyokinetic Figure (33 minutes).*

The nuclear membrane is here disappearing, but still remains at the upper side. The spindle, now clearly apparent, is separated from the remains of the nuclear membrane by a distinct interval.

The chromatin appears both in the form of rods and rings (cf. Text-fig. XIII. C). One of the latter is clearly shown.

## PLATE VII. PHOTOTYPE 26.

*The Karyokinetic Figure (42 minutes).*

The rod-shaped chromosomes are now arranged to form the equatorial plate. The section is extremely thin, and the figure shows clearly the cyto-reticulum and the relation of the astral rays to it (cf. Text-fig. XIV.). In this specimen the entire amphiaster is surrounded by a granular zone, in which the meshes of the reticulum are finer than elsewhere (but this is not always the case). Here and there the astral rays can be traced, as linear rows of granules, directly through this zone into the outer reticulum.

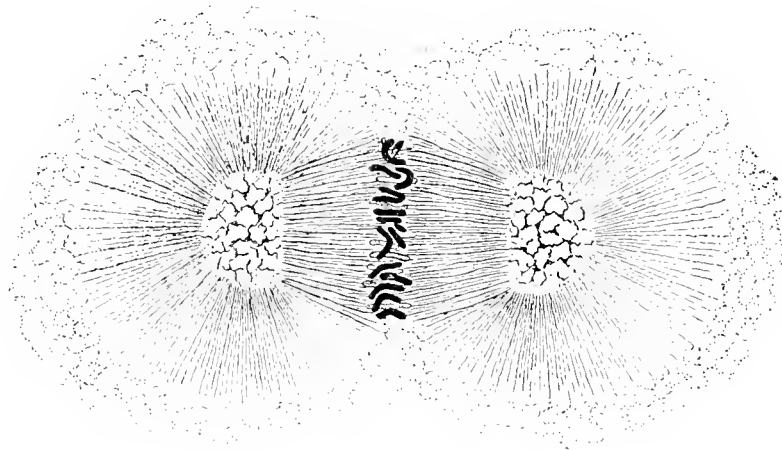


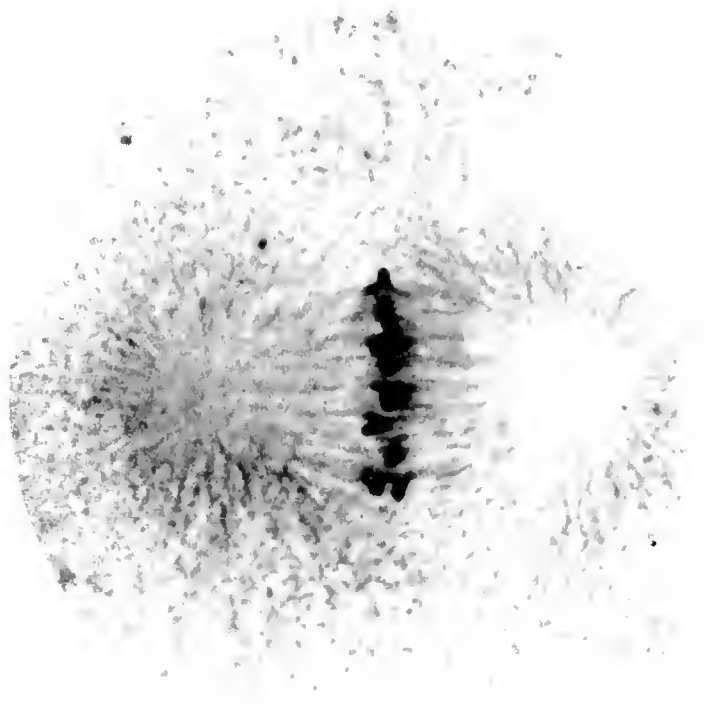
FIG. XIV.

FIG. XIV. — The karyokinetic figure fully developed, with completely formed spindles and asters (1500 diameters). The chromosomes are arranged in the equatorial plane of the spindle to form the "equatorial plate." The centre of each aster is occupied by a reticular centrosphere. The rays are distinctly granular in structure, very closely set, and at their outer ends become continuous with the reticulum. (Phototype 26.)

## PLATE VII. PHOTOTYPE 27.

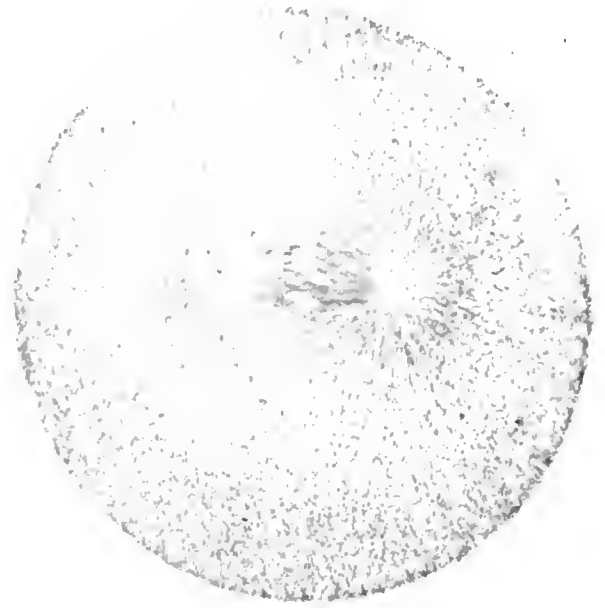
*The Karyokinetic Figure (38 minutes).*

This specimen, slightly earlier than the last, shows more clearly the reticulated centrosphere in the centre of each aster.



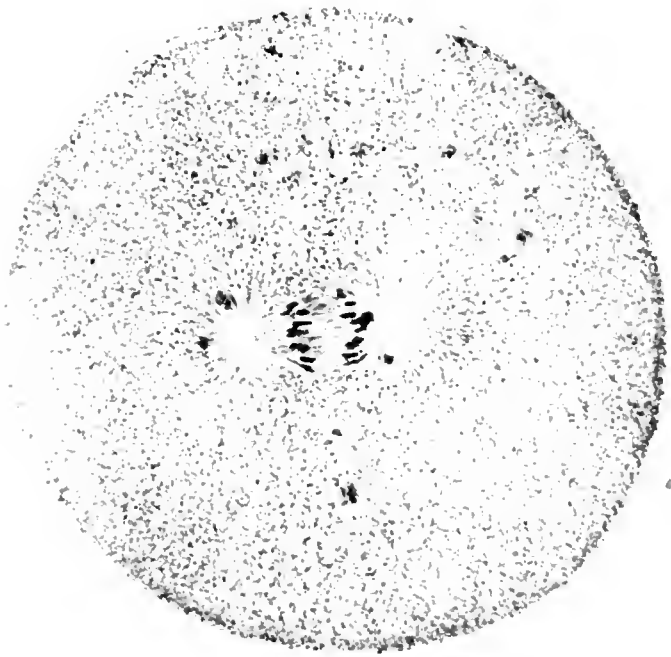
29

METAPHASE ENLARGED 3000 DIAMETERS. SPLITTING AND SEPARATION OF THE CHROMOSOMES (P. 25).



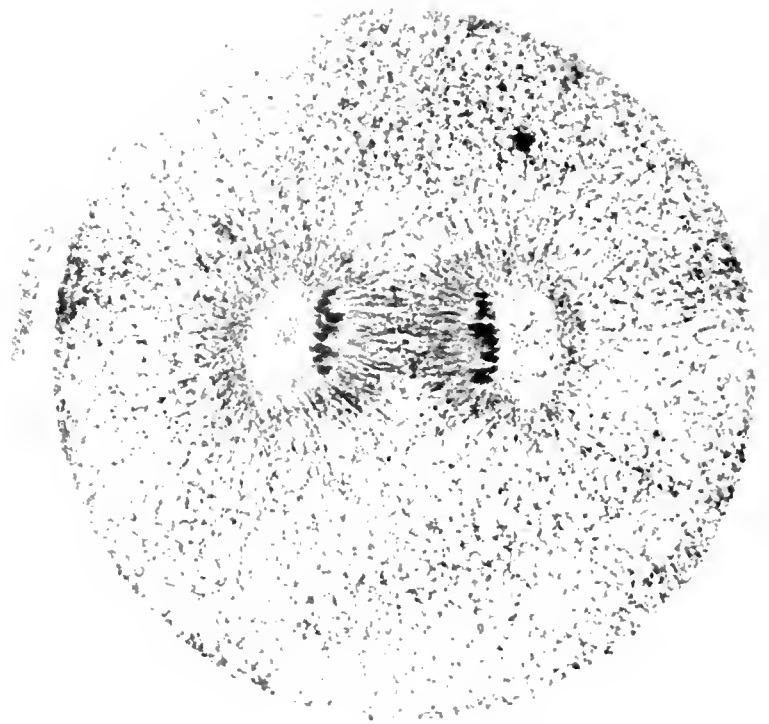
30

EARLY ANAPHASE. CHROMOSOMES SEPARATING. CENTROSPHERES (P. 26).



31

LATER ANAPHASE. SYMMETRICAL GROUPING OF THE DAUGHTER CHROMOSOMES (P. 27).



FINAL ANAPHASE. COMPLETE DIVERGENCE OF THE DAUGHTER CHROMOSOMES (P. 27).



## VI. THE METAPHASE. DIVISION OF THE CHROMOSOMES.

Soon after the stage shown in Text-fig. XIV., the chromosomes become nearly straight and place themselves with their long axis transverse to the long axis of the spindle. As they do so, each of them splits lengthwise into two exactly similar halves, which are destined to pass to opposite poles of the spindle and enter into the formation of the two respective daughter-nuclei. The two halves always begin to diverge first at that end of the chromosome turned towards the interior of the spindle (Text-fig. XV.). From this point the halves seem to be pulled apart in opposite directions, leaving them still attached at the other end, at which point the final separation takes place (Text-fig. XVI. A).

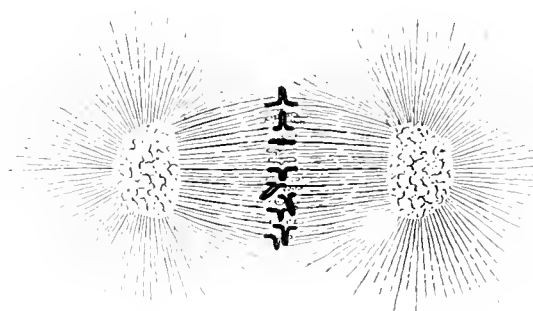


FIG. XV.

FIG. XV.—The metaphase.

All of the chromosomes are split lengthwise. The halves are being dragged apart at the inner end of the chromosome, but remain united by their outer ends. (Phototypes 28, 29.)

## PLATE VII. PHOTOTYPE 28.

*Metaphase. Splitting of the Chromosomes (42 minutes); 1000 diameters.*

This is the same specimen shown in Text-fig. XV. The spindle and asters are well shown—the centrospheres slightly out of focus. The focus is sharply upon the chromosome at the upper edge of the spindle, which is clearly shown in the shape of an inverted Y, the daughter-chromosomes being still united for about one-third their length. Two similar chromosomes, not quite in focus, appear at the lower side, and one or two others near the centre. A more enlarged view of the same equatorial plate is given in the following phototype.

## PLATE VIII. PHOTOTYPE 29.

*The Same Specimen at an Enlargement of 3000 Diameters (focus slightly changed).*

In this figure most of the chromosomes shown in Text-fig. XV. can be individually made out (the illumination employed was such as to sacrifice most of the detail in the achromatic structures). The splitting is clearly shown in the lower right-hand individual.

## VII. THE ANAPHASES. DIVERGENCE AND SEPARATION OF THE DAUGHTER-CHROMOSOMES.

After the division is completed, the daughter-chromosomes have the form of short straight rods, placed with their long axes parallel to that of the spindle. They now rapidly separate, passing along the spindle-fibres until they come into actual contact with the centrosphere (Text-fig. XVII. A). The latter has meanwhile greatly increased in size and has a sharply defined boundary. Its substance is composed of a delicate reticulum, the meshes of which stain red with the double stain, while the astral rays are blue. The latter have now again extended themselves far out into the cytoplasm.

The nature of the force by which the daughter-chromosomes are separated is still in dispute. The view generally prevailing among zoölogists is that of Van Beneden, according to which the astral rays and spindle-fibres are contractile elements analogous to muscle-fibres. By contraction of the spindle-fibres the daughter-chromosomes,

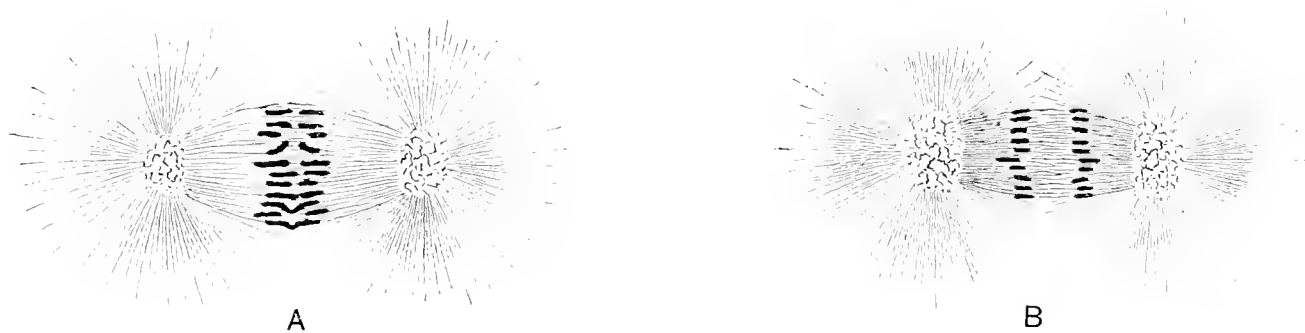


FIG. XVI

FIG. XVI.—The “anaphases”; divergence of the daughter-chromosomes (1000 diameters).

- A. The chromosomes in various stages of separation. In two of them the daughter-chromosomes are still united at one end.  
 B. Divergence of the daughter-chromosomes. (Phototype 31.)

to which they are attached, are believed to be mechanically dragged apart and transported to the spindle-poles. This view is supported by evidence so strong that it can scarcely be escaped in the case of some animal cells. Strasburger and some other botanists, on the other hand, maintain that there is no evidence in its favour in the case of plant-cells and regard the movement of the daughter-chromosomes as perhaps a chemotactic action caused by the formation of special chemical products at the spindle-poles.

As the phototypes about to be described clearly show, the facts in *Toxopneustes* do not accord entirely with either of these views.

## PLATE VIII. PHOTOTYPE 30.

*Early Anaphase, showing especially the Achromatic Structures.*

The daughter-chromosomes (not very sharply shown) are separating. The centrospheres have become sharply defined, and the reticulum is clearly shown. The astral rays are extending.



## PLATE VIII. PHOTOTYPE 31.

*Late Anaphase, showing especially the Chromosomes.*

The section passes slightly to one side of the spindle-axis, and hence does not show the achromatic figure as well as the last or the following. The chromosomes are, however, very clearly shown, and they may be seen to correspond accurately on the two sides, each daughter-chromosome having its fellow on the opposite side (cf. Text-fig. XVI. B). Observe the very considerable variation in size, and also the varying intervals between the halves of each pair. The latter fact shows that the chromosomes move to some extent independently of one another.

Stretching between the two groups of daughter-chromosomes, in the equatorial region of the spindle, may be seen spindle-fibres, known as the "interzonal fibres" ("filaments réunissants," "Verbindungsfasern"), regarding which much discussion has arisen (I.F. in Text-fig. V. C). According to Hermann they are non-contractile and form a "central spindle" along which the chromosomes are dragged, the contractile fibres forming the peripheral portion of the spindle ("Spindle-mantle"). As will appear beyond, there is no ground in *Toxopneustes* for making any distinction between these fibres and the others.

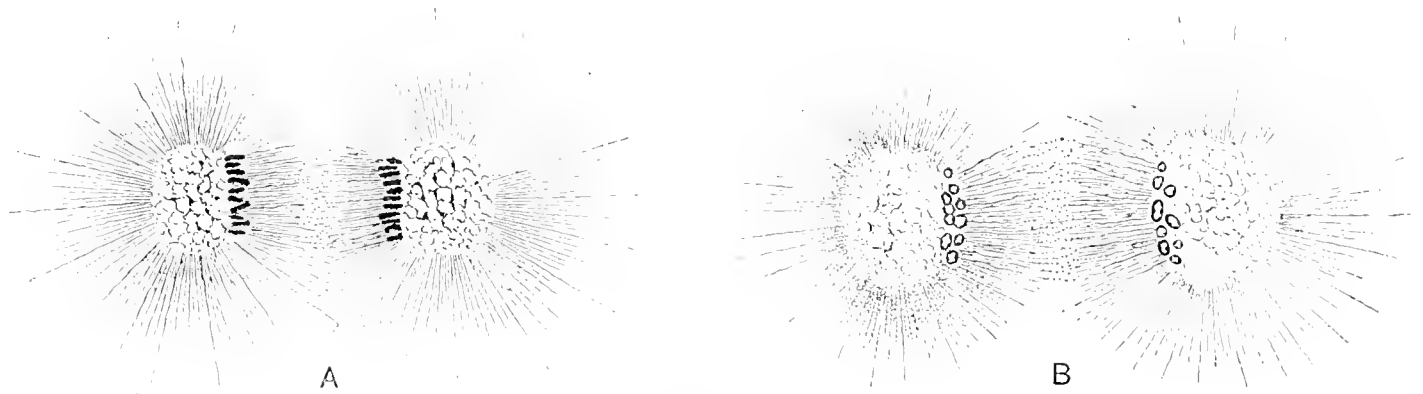


FIG. XVII.

FIG. XVII.—The closing stages of karyokinesis (4000 diameters).

A. Late anaphase. The daughter-chromosomes have passed to the spindle-poles and lie in contact with the reticular centrosphere. Central portion of the spindle breaking up to form the "Zwischenkörper," or mid-body (Phototype 32).

B. The chromosomes have become converted into vesicles. The centrosphere very large, its borders vague and indistinct. (Phototype 34.)

## PLATE VIII. PHOTOTYPE 32.

*Late Anaphase. Complete Divergence of the Daughter-chromosomes.*

The daughter-chromosomes have now passed outwards to the extreme limit of the spindle and lie in contact with the centrosphere. Were their movements due to the action of contractile fibres, the thickened fibres should appear between them and the centrosphere. No such fibres are present, however, and the movements must therefore have some other cause.

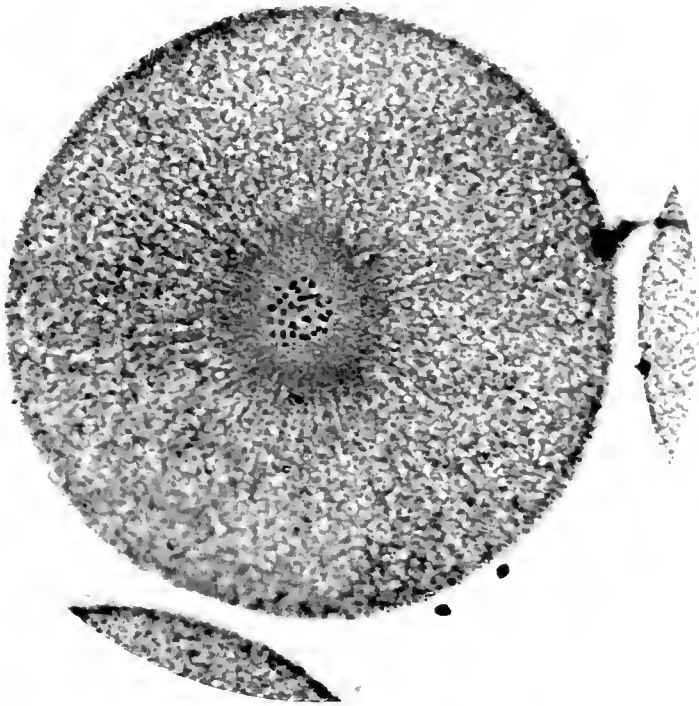
The achromatic figure is now nearly at a maximum. The centrospheres are very conspicuous, and their reticular substance is clearly shown. The aster consists of an inner denser zone, composed of close-set granular rays, and a peripheral looser portion composed of long rays extending far out into the general reticulum, the meshes of which are here very plainly shown.

The equatorial region of the spindle is becoming disorganized, the fibres breaking up into rather coarse blue granules which, at a later stage, form a body known as the "Zwischenkörper," or mid-body.

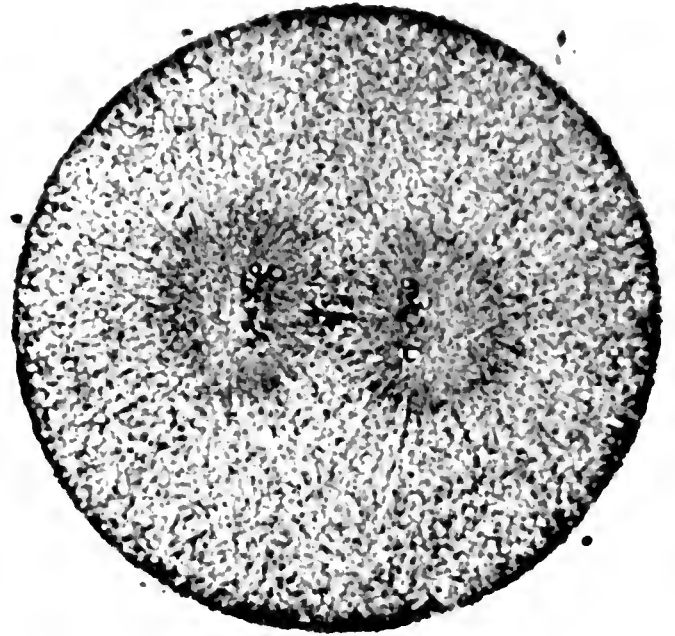
## PLATE IX. PHOTOTYPE 33.

*Cross-section of the Karyokinetic Figure, showing the Chromosomes.*

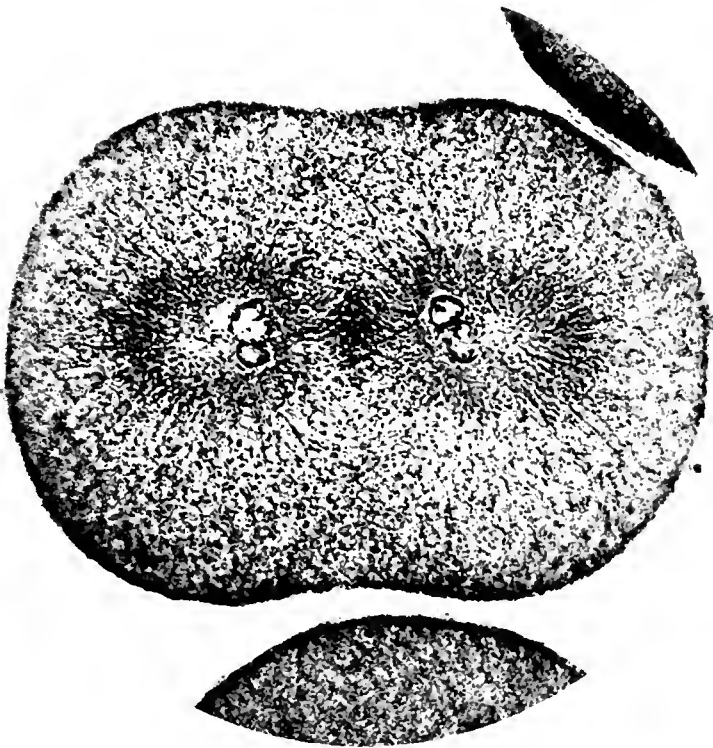
This specimen is of the same stage as the last, but viewed in cross-section (i.e., from the end of the spindle). In the section, 38 chromosomes can be counted, but not more than 30 are in focus. Half of these are of paternal, half of maternal, origin. This view shows the important fact that the chromosomes do not surround the spindle, but extend through its entire diameter. There can, therefore, be no distinction between a "central spindle" and a "spindle-mantle." If two kinds of spindle-fibres be present (cf. p. 27), they must be intermingled. But there is no evidence that the fibres are, in this case, of two kinds.



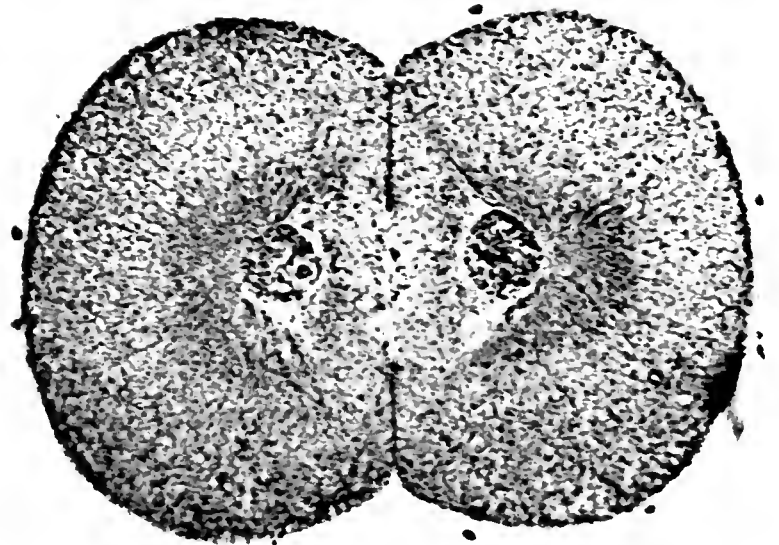
33.  
CROSS-SECTION OF LATE ANAPHASE SHOWING THE CHROMOSOMES (P. 28).



34.  
EARLY TELOPHASE SHOWING THE CHROMOSOMAL VESICLES (P. 29)



35.  
THE BEGINNING OF CLEAVAGE. MIDBODY AND NUCLEAR VESICLES (P. 29)



36.  
CLEAVAGE NEARLY COMPLETED. THE DAUGHTER NUCLEI RE-FORMED (P. 29)



## VII. TELOPHASES. RECONSTRUCTION OF THE DAUGHTER-NUCLEI. CLEAVAGE.

The closing phases of karyokinesis, which differ considerably in different kinds of cells, are very beautifully shown in *Toxopneustes*. The history of the chromatic and achromatic structures may be separately considered.

A. **The Chromatin.**—Immediately after the stage just described, each chromosome is converted into a small sac or vesicle (Text-fig. XVII. B), a clear space appearing in the interior, while the chromatin forms an irregular wall about it. The vesicles then rapidly fuse together, their number becoming progressively reduced until only two or three larger nuclear vesicles are left on either side (Text-fig. XVIII.). The egg now divides into two, and as it does so, the remaining nuclear vesicles unite on either side to form a single daughter-nucleus (Text-fig. XIX.).

The formation of the chromatic vesicles seems to be of wide occurrence, especially in the early stages of development. It is, perhaps, comparable with the secondary splitting of the daughter-chromosomes, observed by Flemming in the heterotypical karyokinesis of the testis-cells in the *Salamander*, and by Van Beneden and Herla in *Ascaris*.

B. **The Achromatic Structures.**—As the chromosomal vesicles are formed, they pass into the outer portion of the centrosphere, which now rapidly changes its character, its outline becoming indistinct, and its staining capacity changing. It no longer stains clear red in the double-stain, but purple, and is filled with blue granules. As the vesicles fuse, and the cell divides, the centrosphere nearly disappears, leaving, however, an irregular and shrunken remnant, which, in some cases at any rate, extends completely around the daughter-nuclei. The aster, as a whole, however, persists.

As the cell divides, the mid-body (cf. Text-fig. XVII.) lies exactly in the line of division as a somewhat lens-shaped mass of blue granules. Upon completion of the division it can no longer be found; and, moreover, the remaining portions of the spindle-fibres completely disappear. The aster is then left (Text-fig. XVIII.) as a horseshoe-shaped body, lying beside the nucleus in each daughter-cell. Soon afterwards it divides into two to form the asters for the succeeding cleavage.

## PLATE IX. PHOTOTYPE 34.

*The Chromosomal Vesicles.*

The central portion of the spindle has broken up into granules (aggregated to form the mid-body in the following stage), the peripheral portion is indistinguishable from the aster, of which it forms a part. Within each aster may be seen a group of minute oval vesicles derived from the daughter-chromosomes of the last stage. The centrospheres are very large, but ill-defined (Text-fig. XVII. B).

## PLATE IX. PHOTOTYPE 35.

*Cleavage.*

The section passes horizontally through the egg at the moment of division. In the centre lies the mid-body, darker and somewhat ill-defined. On either side of this is a group of 2-3 nuclear vesicles formed by the fusion of

the chromosomal vesicles of the preceding stage. The remains of the centrosphere, scarcely defined, surround the nuclear vesicles.

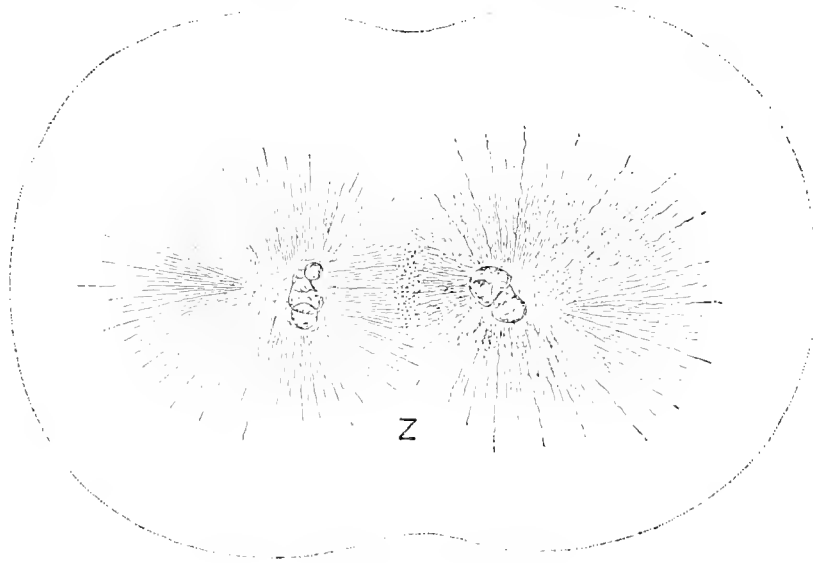


FIG. XVIII.

FIG. XVIII. — The egg beginning to divide (1000 diameters).

The chromosomal vesicles have conjugated, leaving three larger vesicles on one side and two on the other. These vesicles lie in the interior of the shrunken centrosphere. "Zwischenkörper" (above Z) a lenticular mass of blue granules. (Phototype 35.)

PLATE IX. PHOTOTYPE 36.

*First Cleavage nearly Completed.*

The egg has nearly divided into two and the nuclear vesicles have fused on each side to form a rounded daughter-nucleus traversed by a very fine and pale reticulum of chromatin, and still surrounded by the remains of the centrosphere.

The spindle-fibres have entirely disappeared, leaving the aster as a large horseshoe-shaped mass of granular rays extending around the nucleus.

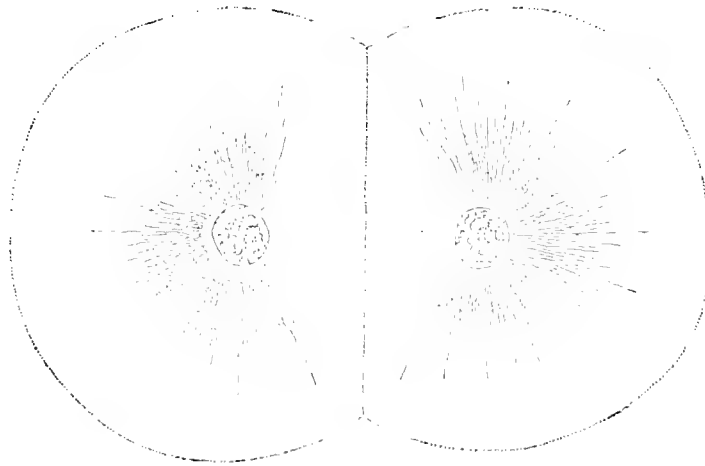
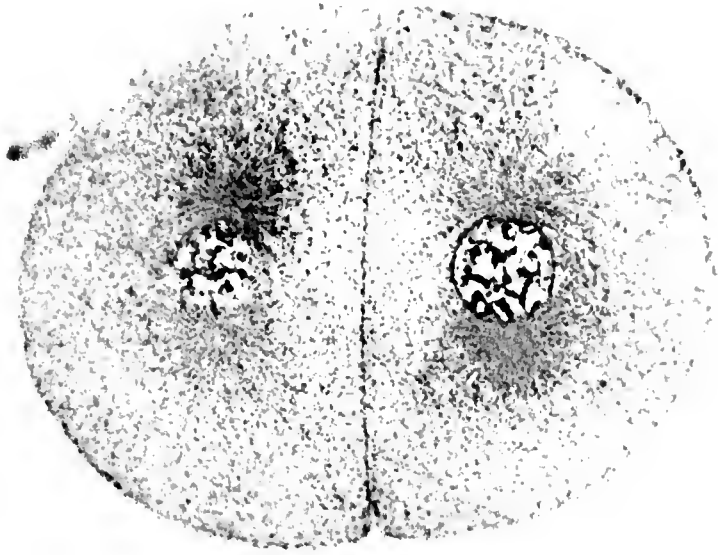


FIG. XIX.

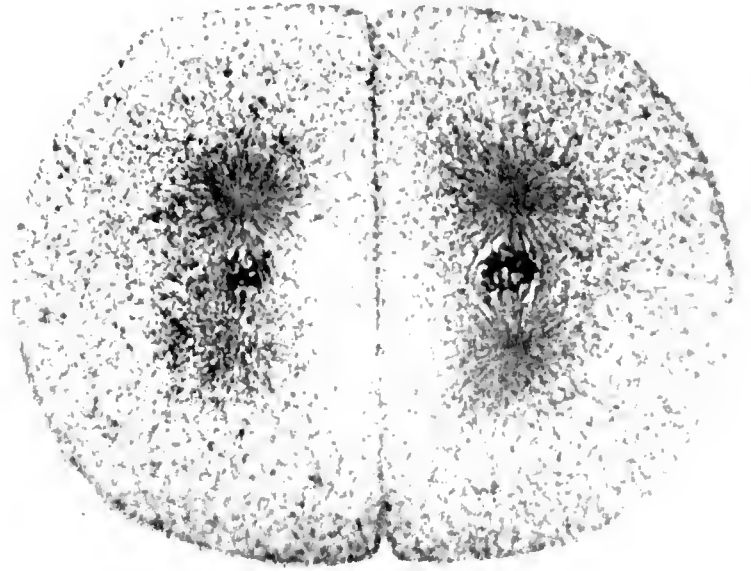
FIG. XIX. — Two-cell stage immediately after division, a few minutes later than the last.

The nuclear vesicles of the last stage have fused to form in each cell a small daughter-nucleus still surrounded by the remains of the centrosphere. The inner portion (towards the division-plane) of each aster, originally a part of the spindle, has disappeared. (Phototype 39.)



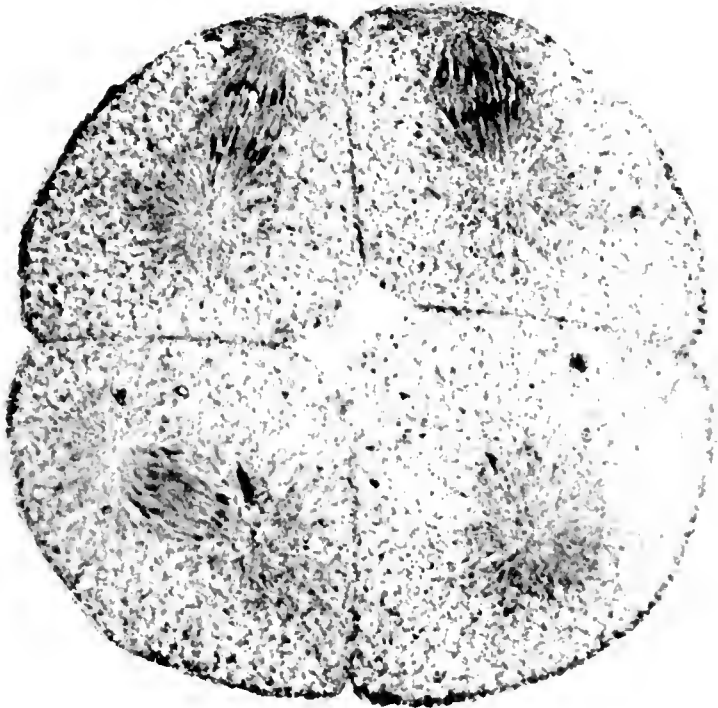
37

TWO-CELL STAGE DURING THE PAUSE, AFTER FISSION OF THE ASTERS (P. 31)



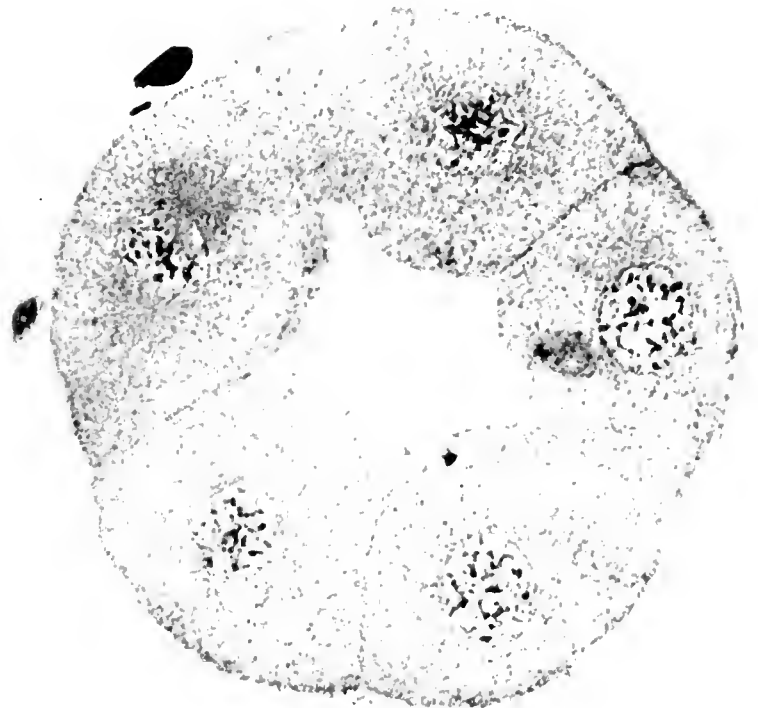
38

PREPARATION FOR THE SECOND CLEAVAGE (P. 31)



39

THE FOURTH CLEAVAGE IN PROGRESS, SHOWING THE MICROMERE-SPINDLES (P. 32)



40

BLASTULA, SIXTEEN-CELL STAGE IN SECTION (P. 32)





## VIII. FROM THE TWO-CELL STAGE ONWARDS.

After completion of the first cleavage each of the two resulting cells or blastomeres passes through a series of changes analogous to those occurring in the undivided egg after the union of the germ-nuclei. In the first place the nucleus greatly enlarges and its staining power gradually increases. In the second place the horseshoe-shaped aster (cf. Figs. XIX. and XII. A) divides into two halves which place themselves at opposite poles of the nucleus,  $90^\circ$  away from the centre of the mother-aster. The astral rays meanwhile become shorter, and the centre of each aster again acquires the capacity of staining light red after double staining with hæmatoxylin and Congo red, while the rays are blue.

The subsequent stages repeat those already described in the undivided egg without essential modification.

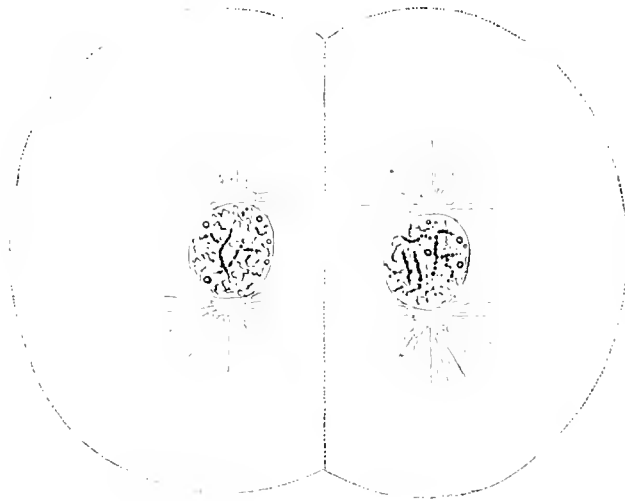


FIG. XX.

FIG. XX. — Two-celled stage during the "pause" or resting-stage.

The nuclei have greatly increased in size and staining power, and contain many chromatic rings besides the reticulum. The aster has completely divided, forming two asters in each cell. (Cf. Fig. XIII. A.) (Phototype 37.)

## PLATE X. PHOTOTYPE 37.

*Two-cell Stage during the Pause.*

The mother-aster has divided into two in each cell, and the nuclei have considerably increased in size and staining power. The position of the asters shows that the plane of the second cleavage, at right angles to the first, is fully predetermined. No trace of the mid-body remains, and the former spindle has been entirely resolved into cytoplasm.

## PLATE X. PHOTOTYPE 38.

*Two-cell Stage preparing for the Second Cleavage.*

A typical karyokinetic figure is forming in each cell at right angles to that of the first cleavage.

## PLATE X. PHOTOTYPE 39.

*The Fourth Cleavage.*

The embryo is here shown in vertical section at the moment preceding the division from 8 to 16 cells. The two lower cells are dividing equally and vertically, one of the amphiasters being seen endwise, the other *en face*. The two upper cells are about to divide unequally to form two smaller cells or micromeres (p. 2, Fig. I). As the phototype shows, the chromosomes are equal on the two sides, but the asters are conspicuously unequal, and in this fact perhaps lies the immediate cause of the inequality of division. Why the aster should be unequal is not known. It is perhaps due to the position of the karyokinetic figure, which, as the figure shows, lies excentrically in the cell.

## PLATE X. PHOTOTYPE 40.

*The Blastula. Sixteen-celled Stage.*

This section shows the blastomeres arranged in a hollow sphere surrounding a central *blastocæl* or *cleavage-cavity*. The nucleus is visible in each cell, and some of them show also the attraction-spheres (asters).





















