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# MORPHOLOGY.



EDITED BY

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WITH THE CO-OPERATION OF

EDWARD PHELPS ALLIS.

*Milwaukee.*

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# CONTENTS OF VOL. XV.

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# JOURNAL

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## A NEW PERIPATUS FROM MEXICO.

WILLIAM MORTON WHEELER.

Dr. Gustav Eisen, of San Francisco, has most generously placed at my disposal all his material — some 87 specimens — of a new species of Peripatus, with permission to describe the animal and the development of its embryos. The specimens were taken by Dr. Eisen during November, 1894, at Tepic, Mexico, at an altitude of 4000 feet. He found them under stones and pieces of wood in a shady spot along a ditch of water flowing from some baths on the outskirts of the town. The animals were killed in a fully extended condition by drowning them in water, to which a few drops of ammonia had been added. They were then carefully hardened in corrosive sublimate.

The species which I take pleasure in naming *Peripatus eisenii*, after its discoverer, is a true neo-tropical Peripatus. With the exception of *P. edwardsii* Sedgwick, from Venezuela, the descriptions of the American species are not very satisfactory. I shall, therefore, make most use of a comparison with this species in the following description of the exterior of *P. eisenii*. Anatomical and embryological details are reserved for future publication.

The specimens vary considerably in length. The youngest individuals, evidently just born, measure from 13 to 20 mm., the adults from 40 to 57 mm. One female only 30 mm. in length contains full-grown pigmented embryos.

Dr. Eisen informs me that the preservation in alcohol has not altered the colors of the animal. These are very variable, the dorsal surface of the body and legs ranging through some four or five shades, — from light pinkish brown in many of the youngest specimens to a dark reddish chocolate color in some of the oldest. Most of them show a distinct darker brown median dorsal stripe, and some have the faint transverse intersegmental stripes indicated in the figure (Fig. 1). Along either side of the mid-dorsal line are seen a number of white dots, which are in reality the unusually large pale papillae seen under a higher magnification in Fig. 8. The ventral surface, too, is variable in its coloration. Some specimens are pale yellow, like Figs. 2 and 5, others are white, still others flushed with pink. The lips of the oral orifice, the oral papillae, and the spinous creeping pads on the feet are paler than other portions of the ventral surface.

The transverse ridges of the integument bear each a single row of papillae. Some of these are enlarged, especially on the legs, and consist of two segments, — a broad basal moiety which is usually conical, although in some cases it approaches the condition called "cylindrical" by Sedgwick[1] in his description of *P. edwardsii*, and a more slender tapering apical moiety, tipped with a spinule and unpigmented. It would seem that the animals have the power of retracting the pale distal moiety with its spinule, but this can only be determined by a study of the living animal.

In the mid-dorsal line the transverse ridges of the integument are interrupted by a delicate but perfectly distinct impressed white line (Fig. 8). The absence of this white line is emphasized by Sedgwick as a diagnostic character of the American, or neo-tropical, species of Peripatus. I find this

[1] Adam Sedgwick, "A Monograph of the Species and Distribution of the Genus Peripatus." *Quar. Journ. Micr. Sci.*, vol. xxviii, 1888. Also in *Studies from the Morph. Lab. Univ. Camb.*, vol. iv, pt. ii, 1888, pp. 147–212, Pls. XIV–XX.

line also in some specimens of *P. trinidadensis* Sedgwick, although it is so much obscured by the shrinkage of the integument that I should have overlooked it had I not previously found it in *P. eisenii.* The impressed line may be somewhat narrower and fainter than it is in the Cape species (*P. capensis* Grube and *P. moseleyi* Wood Mason; see Sedgwick's Pl. XVII, Fig. 10), but its presence in at least two of the American species is sufficient reason for excluding it in future from the diagnosis of the South African division of the genus.

The antennae and oral papillae resemble the corresponding organs of *P. edwardsii.* The jaws, too, resemble the jaws of the Venezuelan species, except that the inner blade bears three teeth before the diastema instead of two. The additional tooth is a smaller second "minor" tooth. The teeth of the series beyond the diastema are less numerous and blunter than they are in *P. edwardsii.* The outer blade of the jaw in *P. eisenii* bears only two teeth, like that of the other species of the genus.

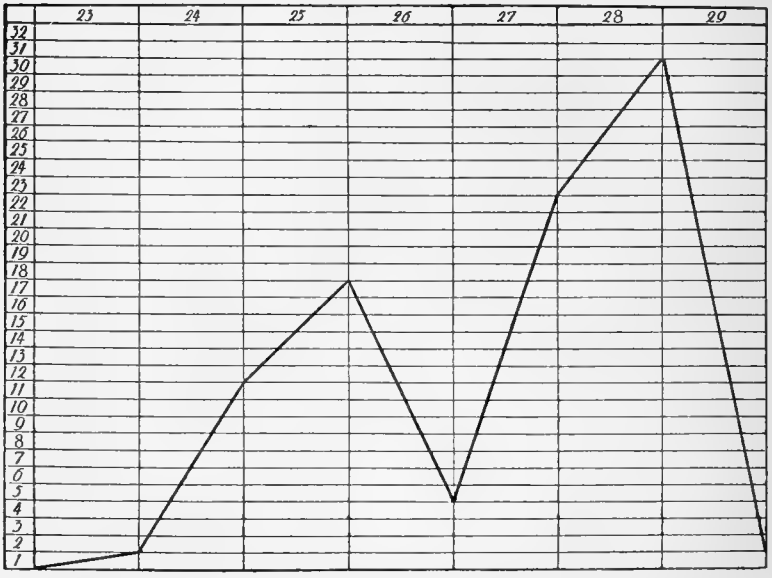
The papillae and folds surrounding the mouth differ from those in the same position in *P. edwardsii.* These differences are best seen by comparing Sedgwick's Pl. XVIII, Fig. 15, with my Fig. 2.

*P. eisenii* resembles the other American species in having a variable number of legs. The legs in all the specimens, old and young, were counted, and were found to vary from 23 to 29 pairs. The extremes were each represented by a single specimen. Four had 26 pairs, 11 had 24, 17 had 25, 22 had 27, and 30 had 28 pairs. The peculiar curve plotted from these data has two summits as shown in the accompanying figure.

This peculiar variation in the number of pairs of legs is not due to growth, as Sedgwick has shown in other neo-tropical species. The animal is born with the definitive number of legs, and no further pairs are added during post-embryonic life. Several of what I take to be just-born young, only 11 to 13 mm. long, have 27 or 28 pairs of legs, whereas several larger specimens, 20 to 28 mm. long, have only 24 or 25 pairs.

According to Sedgwick, the greater numbers are found in the females, the lesser in the males. I have been unable as

yet to find any males in the material. Of 16 adult females containing embryos, 6 had 27, 9 had 28, and 1 had 29 pairs of legs. This would tend to corroborate Sedgwick's statement and to show that the second summit of the curve represents




the usual number in the female, the first the usual number for the male of *P. eisenii.* This point can be determined only after the smaller individuals have been sectioned and their sex ascertained.

The following list gives the numbers of pairs of legs in the better known species of Peripatus, from both hemispheres, as recorded by Sedgwick :

| | |
|---|---|
| *Peripatus brevis* Blainv. S. Africa. | 14 |
| *novae zealandiae* Hutton. New Zealand. | 15 |
| *leuckarti* Saenger. Queensland. | 15 |
| *capensis* Grube. S. Africa. | 17 |
| *balfouri* Sedgw. S. Africa. | 18 |
| *moseleyi* Wood Mason. S. Africa. | 21–24 |
| *chiliensis* Sedgw. Chili. | 19–27 |
| *eisenii* n. sp. Mexico. | 23–29 |
| *peruanus* Grube. Peru. | 29 |
| *demeraranus* Sedgw. Demerara. | 27–31 |

| | | |
|---|---|---|
| *Peripatus trinidadensis* Sedgw. | Trinidad. | 28–32 |
| *juliformis* Gilding. | St. Vincent. | 33 |
| *edwardsii* Sedgw. | Venezuela. | 29–34 |
| *quitensis* Schmarda. | Quito. | 36 |
| *torquatus* Kennel. | Trinidad. | 41–42 |

It will be seen from this list, in which we have a graduated series of forms with legs varying from 14 to 42 pairs, that the Mexican Peripatus has the lowest number of legs of any American species with the exception of *P. chiliensis*.[1]

In the structure of its legs *P. eisenii* resembles the other neo-tropical species. All the feet have four spiny creeping pads, except the last two pairs, which have only three (Fig. 5). The most proximal pad on the antepenultimate pair is very small, so that this pair forms a transition between the feet in front with four pads and the posterior feet with three. According to Sedgwick, only the last pair of feet has three spinous creeping pads in *P. edwardsii*.

The opening of the nephridium on the fourth and fifth pairs of legs in *P. eisenii* differs from that observed in other American species. The position of this opening is shown in Fig. 7. The second pad from the base of the appendage is broken in two, — a short posterior and a much longer anterior piece, — and between them lies the papilla with the nephridial orifice. In *P. edwardsii* the second spinous creeping pad is entire, though somewhat narrowed in the middle; and the nephridial papilla lies between it and the most proximal pad in the middle line of the appendage, according to Sedgwick (Pl. XVII, Fig. 11). In the old-world species figured by Sedgwick (*P. balfouri*, Pl. XVII, Fig. 9, and *P. novae zealandiae*, Pl. XIX, Fig. 21) the nephridial papilla lies in the middle of the proximal pad, which corresponds to the second pad from the base of the appendage in *P. eisenii* and other new-world species.

The position of the nephridial papilla in the middle longitudinal line of the appendage, as represented by Sedgwick for *P. edwardsii*, will not hold good for at least one other American species, *viz.*, *P. trinidadensis*. In the specimens of this species

[1] The number of pairs of legs in this species has not been satisfactorily reported. See Sedgwick, p. 197.

which I have examined the nephridial papilla lies between the most proximal and the succeeding pad, as in *P. edwardsii*, but distinctly nearer the posterior than the anterior surface of the leg.

The pedal grooves are conspicuous on the inner ventral surfaces of all the legs in *P. eisenii*. They are slit-shaped, with thickened, somewhat folded lips. None of the specimens show conspicuous tubercles behind the grooves of some of the posterior legs, like those which have been found in the males of *P. edwardsii*.

The anus is terminal; the reproductive orifice is in the mid-ventral line, between the penultimate pair of legs, as in other American species.

HULL ZOÖLOGICAL LABORATORY, UNIVERSITY OF CHICAGO,
February 28, 1898.

## EXPLANATION OF PLATE I.

FIG. 1. — Dorsal view of a full-grown, rather dark-colored female *Peripatus eisenii* n. sp., magnified about $3\frac{1}{2}$ diameters.

FIG. 2. — View of ventral surface of the head of same specimen enlarged.

FIG. 3. — Outer blade of jaw.

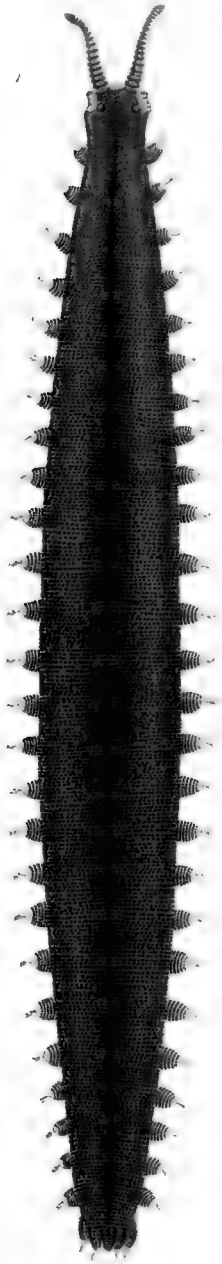
FIG. 4. — Inner blade of jaw.

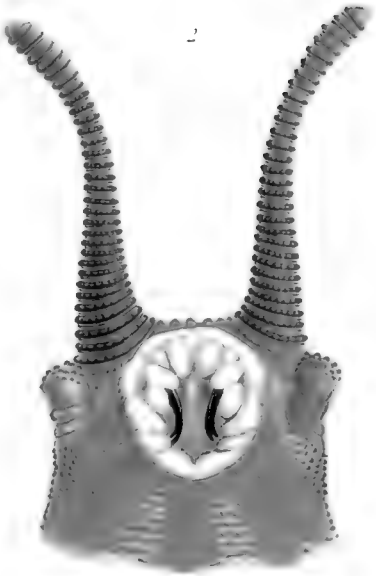
FIG. 5. — View of ventral surface of posterior end of the specimen represented in Fig. 1.

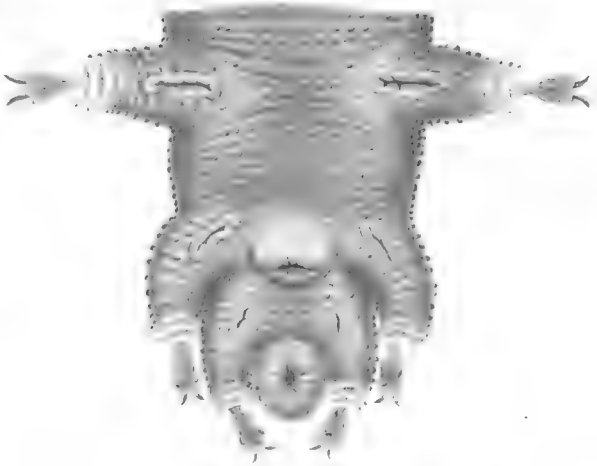
FIG. 6. — Lateral view of one of the feet.

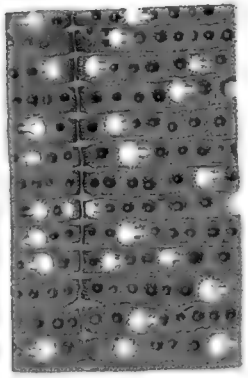
FIG. 7. — Ventral view of the fifth leg, showing the position of the nephridial papilla.

FIG. 8. — Piece of integument, showing the mid-dorsal "white line" interrupting the papillated ridges of the integument.









# THE GERM-RING IN THE EGG OF THE TOAD-FISH (BATRACHUS TAU).

LOUISE B. WALLACE.

SMITH COLLEGE, NORTHAMPTON, MASS.

SINCE the Toad-fish has a number of characteristics peculiar to itself, it is natural to expect that it would differ from the ordinary teleost in its mode of development. The marked resemblance of the egg to that of Elasmobranchs has already been noted,[1] but the formation of the germ-ring has been left an open question. My observations were made during the summers of '95 and '96, under the direction of Dr. Whitman, at the Marine Biological Laboratory, Woods Holl, Mass.

It gives me great pleasure to express my indebtedness also to Dr. Cornelia M. Clapp for many helpful courtesies.

After the middle of June most of the material found in nests in Buzzards Bay was in an advanced condition, and it was necessary to resort to artificial fertilization. Eggs were fertilized by hundreds, covered with sea-water in shallow dishes, and studied from the earliest stages. The egg of Batrachus is 5 mm. in diameter, being much distended with yolk, and is, when deposited, attached to some foreign body by means of an adhesive disc. The blastoderm, in encompassing the yolk, is spread out into a cap of extreme tenuity, requiring delicate treatment. After repeated effort the paraffin method was given up and good results were obtained by use of the celloidin method with either Hermann's fluid or Flemming's fluid as fixing reagents.

Not until the fourth or fifth day after fertilization does a distinct axial thickening appear, with oftentimes a slight, marginal notch at the embryonic pole, and there is no marginal thickening around most of the blastoderm (Pl. II, Fig. 6). A median, longitudinal section is shown in Pl. III, Fig. 1. In

[1] Cornelia M. Clapp, "Some Points on the Development of the Toad-fish (Batrachus tau)." *Journ. of Morph.*, vol. v, No. 3.

the extra-embryonic region the ectoderm is two cells deep, with no peripheral thickening ; while in the embryonic region there is a centripetal growth of cells, thickest near the margin and thinning out anteriorly until some of the cells appear to be lying loose on the periblastic floor. A cross-section, passing through the axial thickening, shows that this tongue of cells also thins out laterally (Pl. III, Fig. 2). Very soon after the germ-ring attains its maximum development (Pl. II, Fig. 7) it begins to decrease. This decrease is shown in an enlarged view of a later stage, Pl. III, Fig. 9, in which the ring is narrow at the anterior pole, gradually broadening toward the posterior or embryonic pole. Cross-sections of the rim at the cardinal points reveal an interesting modification of the germ-ring in the ordinary teleostean egg. In a section at the anterior pole no invagination obtains, but rather a centripetal proliferation of cells from the ectoderm (Pl. III, Fig. 3). In Professor Wilson's paper on the Sea Bass [1] he says: "The peripheral part of the blastoderm, both where there is a large Randwulst and none at all, is an undifferentiated area, and the germ-ring consequently starts at some distance from the extreme edge of the blastoderm." If we follow this interpretation of terms, we have in Batrachus no germ-ring, as there is no under layer of cells differentiated from the rest of the blastoderm. A section through the lateral region more strongly expresses the fact that there is no invagination, the blastoderm being actually thinner at the periphery, as shown in Pl. III, Fig. 4. Here, also, we find no distinct under layer, but a few loose cells which are budded off centripetally from the slight peripheral thickening. As the occurrence of these loose cells is constant, might they not represent the distinct layer found in other forms? Passing to the posterior pole, a section is shown through the longitudinal axis of the embryo (Pl. III, Fig. 5). Here is a decidedly invaginated appearance, but no real invagination, so far as can be judged from a study of successive stages. The appearance may be due to a rapid proliferation of cells both centripetally and dorso-ventrally (*cf.* Pl. III, Fig. 1), and also to the growth

[1] Henry V. Wilson, "The Embryology of the Sea Bass (Serranus atrarius)." *Bulletin of the U. S. F. C.*, vol. ix. For 1889.

of the ectoderm over the yolk. The ectoderm is sharply differentiated from the ingrowing tongue of cells, especially at the periphery. From this time the ring becomes less and less pronounced. In surface views of the stage shown in Pl. II, Fig. 8, a little irregular thickening can be seen at the anterior pole, and in sections a few scattered cells are found lying beneath the thin, flattened ectoderm (Pl. III, Fig. 6). In some sections not even this much of the thickening remains, as the cells occur in patches. In the lateral region the reduction is not yet carried so far (Pl. III, Fig. 7). Sections through the rim of the stage shown in Pl. II, Fig. 9, have no thickening even in the lateral region, while the tongue of cells at the embryonic pole is steadily lengthening.

In Ctenolabrus Dr. Whitman finds that there is "a plain rolling under or involution as an initiatory step in the formation of the ring," but believes that the process is more correctly described as "an ingrowth due both to a rapid multiplication of the cells and also to the centrifugal expansion of the ectoderm." At the posterior margin "the inrolling portion presents a strongly voluted outline, while at the anterior border it is much more feebly expressed."[1] In Batrachus, around most of the margin there is found simply "the initiatory step," and even that lacks the voluted outline, except at the embryonic pole. The loose cells budded off from this small peripheral thickening represent, I believe, a true germ-ring. In the Sea Bass, Professor Wilson finds, at the embryonic pole, an apparent invagination caused by a centripetal growth of cells, and forming a Randwulst from which cells are proliferated centripetally to form a germ-ring. "Round the rest of the edge the ingrowth is likewise, at least in most places, preceded by the formation of a Randwulst, which, however, is inconspicuous."

From the stage given in Pl. II, Fig. 9, down to the closure of the blastopore at a distance behind the embryo, there is an apparent marginal thickening visible even in the living egg (Pl. II, Figs. 3–5). In specimens killed in Perenyi's fluid, a distinct

[1] Alexander Agassiz and C. O. Whitman, "On the Development of Some Pelagic Fish Eggs." Preliminary notice. *Proceedings of the American Academy of Arts and Sciences*, vol. xx.

opaque rim is noticeable, while in preparations with osmic acid, the rim becomes much darker than the rest of the blastoderm. By the study of surface mounts and from sections, this thickness was found to be due to the greater thickness of the periblast in that region, and also to the accumulation of huge periblastic nuclei. The presence of oil globules increases the effect, especially in the living egg.

Pl. II, Fig. 10, is a reproduction of Dr. Clapp's Fig. 1, d. She says: "In Fig. 1, d, this notch is seen at a little distance behind the embryo; a shadowy connection may be traced between the germ-ring and the embryo." While at this time only the *appearance* of a germ-ring exists, the "shadowy connection" between the "germ-ring" and the embryo has a more substantial basis. The same stage is given in Pl. II, Fig. 5. A longitudinal section through the embryo and the margin of the blastopore is shown in Pl. III, Fig. 8. A few cells, marked "m," are seen lying beneath the ectoderm and reaching from the posterior end of the embryo almost to the lip of the blastopore. Sections all around the blastopore show no thickening except of periblast.

*Summary.* — In the egg of Batrachus there is a centripetal growth of cells at the embryonic pole, the ingrowth having a voluted outline in sections. Around the remainder of the blastoderm there is not even the appearance of an invagination, but only a slight thickening due to an ingrowth of cells from the ectoderm, and a few loose cells which may represent a true germ-ring found as a layer in ordinary forms. The peripheral thickening gradually fades out, first at the anterior pole, until the last remnant is found in a few cells lying beneath the ectoderm, forming a linear streak from the posterior end of the embryo to the lip of the closing blastopore. In the gradual disappearance of the thickening, beginning at the anterior pole and continuing on either side toward the posterior pole, accompanied by the lengthening of the embryo, we see a highly modified form of concrescence.

Woods Holl, Mass.,
September, 1896.

## EXPLANATION OF PLATE II.

FIG. 1. Ovum with blastoderm covering $\frac{1}{3}$ of yolk surface. × 9.

FIG. 2. Ovum with blastoderm covering $\frac{1}{4}$ of yolk surface. × 9.

FIG. 3. Ovum with blastoderm covering over $\frac{1}{2}$ of yolk surface. × 9.

FIG. 4. Ovum with blastoderm covering nearly $\frac{3}{4}$ of yolk surface. × 9.

FIG. 5. Ovum near the time of the closure of the blastopore. × 9. *a.d.* = adhesive disc; *l.* = lip of blastopore.

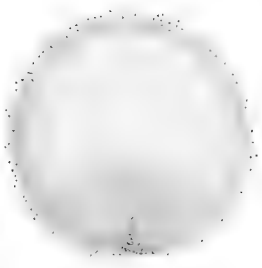
FIG. 6. Blastoderm of an earlier stage than Fig. 1.

FIG. 7. Blastoderm with maximum development of germ-ring. × 16. *g.r.* = germ-ring; *ant.* = anterior; *per.n.* = periblast nuclei.

FIG. 8. Blastoderm of later stage than Pl. III, Fig. 9. × 16.

FIG. 9. Blastoderm of a slightly earlier stage than that of Fig. 3.

FIG. 10. Reproduction of Dr. Clapp's figure showing "shadowy connection," *m.*, between "germ-ring" and embryo.



## EXPLANATION OF PLATE III.

FIG. 1. Longitudinal median section of blastoderm shown in Pl. II, Fig. 6. × 100. *per.* = periblast.

FIG. 2. Cross-section of the same. × 100.

FIG. 3. Cross-section of rim at anterior pole of stage shown in Pl. III, Fig. 9. × 160.

FIG. 4. Cross-section of rim in lateral region of stage shown in Pl. III, Fig. 9. × 160.

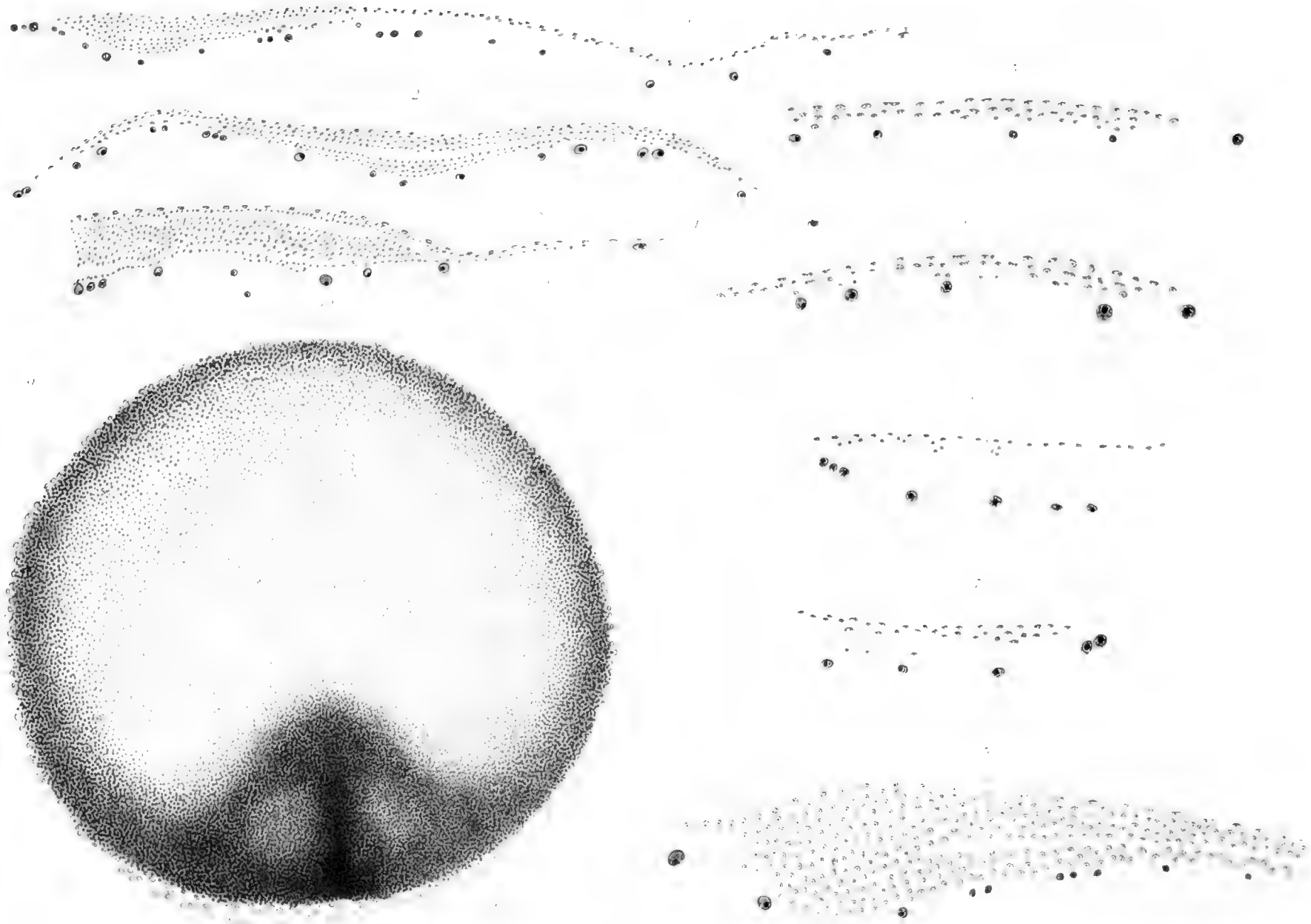
FIG. 5. Longitudinal section through axial thickening of stage shown in Pl. II, Fig. 9. × 160.

FIG. 6. Cross-section of rim at anterior pole of blastoderm shown in Pl. II, Fig. 8. × 160.

FIG. 7. Cross-section of rim in lateral region of blastoderm shown in Pl. II, Fig. 8. × 160.

FIG. 8. Longitudinal median section through embryo and lip of blastopore at stage shown in Pl. II, Fig. 5. × 160. *K.v.* = Kupffer's vesicle; *l.* = lip of blastopore.

FIG. 9. Enlarged view of blastoderm in which the germ-ring is beginning to disappear at the anterior pole. × 45. (Drawn by Mr. Hayashi.)



# THE METAMERISM OF NEPHELIS.

## *A CONTRIBUTION TO THE MORPHOLOGY OF THE NERVOUS SYSTEM, WITH A DESCRIPTION OF NEPHELIS LATERALIS.*

CHARLES L. BRISTOL.

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TABLE OF CONTENTS.



## INTRODUCTION.

THE work which forms the basis of the present paper was begun in 1891 at Clark University, Worcester, and continued at the Marine Biological Laboratory at Woods Holl and Chicago University, and I wish to acknowledge here my indebtedness to the authorities of these institutions for the facilities and the Fellowship privileges granted to me. To Professor Whitman, at whose suggestion I began the investigation, I am deeply indebted for aid and encouragement and for many courtesies extended to me. I am under obligations to Dr. Wm. M. Wheeler

for aid and advice, and to Prof. S. A. Forbes, of the University of Illinois, for the privilege of examining the specimens of Nephelis collected by him under the auspices of the U. S. Fish Commission in the Yellowstone region in Wyoming.

## Historical.

In 1767 Linné enumerated nine species of leeches in one genus, Hirudo. This classification was followed by most later authors, for example, Cuvier, Blumenbach, Carena, and Dumeril, until about 1817, when Savigny, in his *Système des Annelides*, announced the separation of Linné's genus into seven genera. The name Nephelis appears in this work for the first time, although Oken set this leech apart from Hirudo in 1815 under the name Helluo, which genus was to include all fresh-water leeches not provided with jaws. In 1818 Lamarck, at the suggestion of Blainville, proposed the name Erpobdella, which Blainville (1828) urged for acceptance because it contained the descriptive part "bdella." In 1826 Moquin-Tandon adopted Savigny's name Nephelis and continued it in the second edition of his *Monographie des Hirudinées* (1). The name has since become generally accepted, notwithstanding the fact that Oken's Helluo holds priority and Lamarck's Erpobdella is more descriptive.

The first description of Nephelis in America was made by Thomas Say (2) in 1824 under the name of *Hirudo lateralis*. In 1872 Verrill (3) changed this to *Nephelis lateralis*, which, for reasons given in another part of this paper, I have given to the leech I have studied.

## Methods.

The leeches are easily kept in aquaria, for which I used the low glass dishes known as crystallizing dishes, or white earthen-

[1] A. Moquin-Tandon: Monographie de la famille des hirudinées. Paris. 1846.

[2] T. Say: Major Long's Second Expedition to the Source of St. Peter's River, vol. ii, Appendix to the Natural History. 1824. (Republished in *Diesing's Système Helminthologique*, vol. i.)

[3] A. E. Verrill: "Synopsis of the North American Fresh-water Leeches." U. S. Fish Commissioner's Report for 1872–74. (Refers to the *American Journal of Science*, vol. iii, 1872.)

ware cooking dishes, known to the trade as nappies. In some instances I supplied the aquaria with a layer of mud and bottom *débris*, together with a few plants such as Ceratophyllum or Valisneria. When such an aquarium is covered with a glass plate it will keep fresh and clean for a long time and will furnish considerable food for the leeches. Generally, however, I used the plain dish, cleaning out the *débris* and slime and changing the water when necessary. I fed chopped fresh-water clams, but I do not doubt that salt-water clams will serve as well. I have kept individuals for over a year in normal condition and have raised many young under these conditions. When they are first transferred to the aquarium it must be covered for a day or two, to prevent escape. For superficial examination the leeches were killed in very dilute chromic acid, ⅙ to ⅓ per cent solutions. There is one period just before the acid penetrates very deeply when the surface markings stand out very clearly. The leeches usually extend themselves very well, and if killed in a wax tray they may be guided by pins. The best medium for histological details is a ¼ to ½ per cent solution of chromic acid, allowed to act for at least 24 hours. The stains used were borax carmine, Delafield's haematoxylin, and Bizzozero's picro-carmine. The macroscopic characters of the nerve chain were studied from maceration preparations. The leech is killed in a 20 per cent solution of nitric acid and left in it for from 24 to 36 hours, or until the skin and muscles can be easily removed with a porcupine bristle or a glass rod drawn out to a point. These were all carefully dissected away, leaving the chain entire. After thorough washing, the chain may be slightly stained in borax carmine and mounted in glycerine.

A number of details were worked out by the use of Haller's fluid. For example, the head was cut off, slit open on the ventral or dorsal side as wished, and killed in Haller's fluid while it was flattened under a piece of glass. After two days the specimen was transferred to glycerine.

The method that has given me the best results for nerves and sense organs is a gold chloride process kindly given me by Miss Julia B. Platt. It is so simple, so sure, and so exqui-

sitely delicate in some of its effects that it deserves extended use. It may be used with equal success on vertebrate or invertebrate, adult or larval tissue. It must be adapted to the tissue studied ; but this can easily be done after a few experiments. The formic acid appears to be the variable factor, and upon its strength and the time it acts depends the measure of success. I give the procedure applicable to Nephelis.

The leech is killed in a 10 or 15 per cent solution of formic acid, left from 5 to 10 minutes, and then put without washing into a 1 per cent solution of gold chloride for 25 minutes. From this it is transferred, without washing, into a large volume of 1 per cent formic acid, and left for 12 or 18 hours, or until reduction has taken place. It is next washed, passed through the alcohols to chloroform, and then imbedded in paraffin. The sections were cut 18 micra thick. The specimen will appear a rich purple when the reduction has taken place under the best conditions. The precautions are : to use small pieces of material, not thicker than 5 mm., to avoid maceration by reducing the strength of the formic acid and the time of action. My solutions were all well sunned, but no especial precautions were observed.

In tracing out the innervation of the somites it was necessary to examine long, continuous series of sections, and sometimes it was necessary to check results found in one somite by comparison with the next somite. The following method was used which would apply to other purposes. An ordinary library reference card, about 8 cm. by 10 cm., is ruled so as to include as many small rectangles in the same number of rows as the slide to be examined contains sections. The unused margin serves for making notes. An ordinary check mark denotes that the section occupying the same place on the slide that the rectangle does on the card has been examined but does not contain the element under examination. Initials, symbols, different colored pencils, etc., may be used to indicate various details, and each card is numbered the same as the slide. After a number of slides have been carefully plotted in this manner, the cards may be arranged in series and studied as a map. It furnishes an excellent reference-card system for any set of

serial sections, and permits a rapid glance at the order of sequence of any character in different somites or individuals.

## Systematic.

Nephelis differs from nearly all other leeches in the external topography of the somite. While the somite in the Hirudinea, as a group, is characterized by prominent sense organs on the first ring, in Nephelis these are conspicuously absent, save on a few segments near the anus and in rare instances on a few rings near the mouth. The absence of these characters has compelled investigators to resort to other criteria for the determination of species, such as color markings, and the occurrence of four stripes of pigment on the dorsal side is sufficiently well marked to furnish a criterion of generic, if not of specific, value. In Europe the only well-established species is *N. octoculata* Bergmann. Blanchard (4) says: "Jusqu'a Savigny, la seule espèce admise sans contest était la *N. octoculata* Bergmann : Savigny a distingué plusieurs espèces basées exclusivement sur les differences de coloration : mais aucune de ces espèces nominale n'est representée et n'est surement reconnaisible. En outre de la *N. atomaria*, nous croyons pouvoir séparer de l'ancienne *N. octoculata* plusieurs autres formes spécifiques bien distinctes."

Moquin-Tandon in the first edition of his monograph accepts the description given by Carena for *N. atomaria* as a species, but in the second edition lowers it to the rank of a variety of *N. octoculata.*

The first mention of the genus in this country that I have found was made by Thomas Say (2) in 1824 under the name of *Hirudo lateralis*, and this was changed by Verrill (3) in 1872 to *N. lateralis.* Leidy (5) described a form (1870) under the name *N. marmorata.* Verrill describes four species of Nephelis found in the United States and says concerning three of them:

[4] R. Blanchard: "Courtes notices sur les Hirudinées, III. Description de la Nephelis atomaria Carena." *Bull. de la Soc. Zool. de France*, tome xvii, p. 165, 1892.

[5] Jos. Leidy: "Description of Nephelis punctata." *Proc. Acad. Nat. Sci. of Philadelphia*, p. 89, 1870.

"When a larger series of living specimens from various localities can be studied, the three preceding forms (*N. lateralis*, *N. quadristriata*, *N. marmorata*), admitted here as distinct, may prove to be mere varieties of one species, no less variable than *N. vulgaris* of Europe." The fourth species, *N. fervida*, is described from specimens taken from Lake Superior and has eight ocelli. I have not collected a Nephelis answering to this description.

The genus is widely distributed in the United States. My own collections have been made in Massachusetts, Connecticut, Illinois, New York, and South Dakota. I have received specimens from Mr. A. J. Hunter, of Toronto, collected near Toronto, Can., and Professor Forbes, of the University of Illinois, kindly loaned me for examination the specimens of Nephelis collected by him in the Yellowstone region in 1890. Verrill records collections from Maine, Massachusetts, Connecticut, New Jersey, Wisconsin, Nebraska, Colorado (at an elevation of 9000 feet on Longs Peak), and from the waters of Lakes Superior and Huron. The area included covers about 35 degrees of longitude and 10 degrees of latitude; it embraces the Atlantic slope, the Great Lake Region, the Missouri Valley, and the Rocky Mountains.

Investigations on my own collections lead me to agree with Savigny, Moquin-Tandon, and Verrill that it is difficult to distinguish species by the criteria used by them, color and color markings, and to disagree with the methods and results, published by Lindenfeld and Pietruszynski (6), who rely on these features exclusively. My first attempts to classify the specimens which I collected were naturally based on the descriptions given by previous investigators, but it proved so difficult a task to determine what value to place on the various statements of color, and so many of my specimens could with equal propriety be placed in either of two or three categories, that it became evident that some different method of diagnosis would be necessary. The necessity of going beyond color markings was plainly shown by the following experiments.

[6] Von Lindenfeld und Pietruszynski: "Beiträge zur Hirudineen fauna Polens." Reviewed by Nusbaum. *Biol. Centralblatt*, Bd. xii, p. 55, 1892.

I attempted to separate all the individuals collected from one locality near Worcester, Mass., according to color and color marks. I provided five aquaria and sorted each lot as I collected them until the whole number of individuals exceeded one hundred and twenty-five. It was very evident at a glance that the leeches in the first aquarium were light colored, and that those in the fifth were dark colored, but it was impossible to divide them so that each aquarium should be free from transitional forms. I repeated the effort on my collections from Wolf Lake, near Chicago, Ill., and with like results. The very light and very dark individuals were about equally rare, while the great bulk of each lot was made up of leeches varying in shade but having the same stripes more or less distinctly accented according to the amount of pigment present. These trials led me to adopt the method proposed by Whitman (7) and used by Blanchard (4). The method consists in determining the number of rings in the entire body and the limits of each somite. The first ring of each somite in the Hirudinea bears eight sense organs on the dorsal side, as Whitman has shown, four of which are serially homologous with the eyes.

The typical somite of Hirudo contains five rings. This number holds good throughout the middle body region, but falls to three towards the two ends, then to two, and finally to one. The amount and the manner of reduction vary in different genera, but are constant in any given genus. In Nephelis, also, the typical somite has five rings, but the limits of the somites and the number of rings in the terminal ones are not readily determined by the arrangement of the sensillae, for with certain exceptions mentioned hereafter these appear about equally prominent on every ring throughout the entire body. My first attempts to determine these points by means of the sensillae failed; I succeeded later in the following way.

When Nephelis is thrown into weak chromic acid, — 1/6 to 1/3 per cent solution, — there soon comes a time when the sensillae stand out with perfect distinctness; later the contrast in color between them and the surrounding surface becomes

[7] C. O. Whitman: "The External Morphology of the Leech." *Proc. Am. Acad. of Science*, vol. xx, p. 76, 1884.

less and less marked. At the time of greatest distinctness one may see that the sensillae are rather more strongly marked on the terminal somites, especially those at the hind end. It was here that I was able to find a starting point for determining the external metamerization. The 97th ring (Pl. VI, Fig. 3) was strongly marked with sensillae, and between the 96th and 97th rings were found the pores of the 17th and last pair of nephridia. These two conditions gave me a starting point from which I could fix with certainty the limits of the somites towards the anterior until the reduced somites of the eye region were reached. The nephridial pores were used as the limiting marks of the somite forward to the first pair of pores which lie between the 16th and 17th rings. From this point forward the sensillae aided somewhat, but the final results were based on the distribution of the nerves.

A careful reëxamination of my material now showed that, with one exception, I had collected or examined but one species of Nephelis. The exception was found among the leeches collected by Forbes in the Yellowstone region, and while the differences are such that I feel warranted in suggesting that proper study may show them to belong to another species, I could not, from the specimens at hand, determine this point.

The common species of Nephelis found east of the Rocky Mountains is the one that I have used in my investigations. The names adopted by Verrill (3) must, as he prophesies, be abandoned, and the name *Nephelis lateralis* be retained for this species so widely distributed over the United States.

## Description.

The size of the sexually mature adult varies from 4 cm. to 10 cm. at rest. Anterior to the sexual openings the body tapers gradually to the mouth ; posterior to them the body continues about the same size until a little in front of the anus, where it narrows to the sucker. The transection of the body is lenticular, though in the pre-clitellar region it approximates a circle. The body flattens in swimming as it does in Macrobdella and Hirudo.

The color of the adults varies from a light chocolate brown free from any mark of pigmentation to almost a coal black free from any light areas. Between these extremes of very light and very dark all gradations of color and varieties of pigmentation may be found in individuals collected in the same pond or stream. The very light adults are comparatively rare, while among the young smaller individuals unpigmented specimens are quite common. The very dark adults are about as frequent as the very light adults, while a young dark individual is very rare. Most of the individuals that I have collected would fall into two sorts : those in which the pigmentation is diffuse, varying only in intensity through many shades, and those in which the pigmentation is arranged to a greater or less degree in longitudinal stripes. I have collected three individuals that showed definite pigmentation on the first ring of each somite, such as Blanchard (4) describes as constant for *N. octoculata.* Two were from Coonamasset pond near Woods Holl, and one was from Wolf Lake near Chicago. Other specimens with the diffuse type of pigmentation have shown a slightly accented color on the first rings of some of the somites, but not to the extent of defining all the somites. The ground color is either a light mahogany brown or a pale plumbeous gray. This may be observed on the ventral side, which is usually free from pigment. The color of an individual depends upon the amount of dark opaque pigment present either as small granular particles or as highly branching pigment cells. If the view of Graf (8) is correct, that the chloragogen cells wander into the epidermis and there break up, leaving their remains as pigment particles, then the wide variation in individuals taken from the same locality may be explained as individual variations in the manner of excretion.

It is interesting to note that the stripes of color so common in Nephelis lie in the lines of least resistance for wandering chloragogen cells. A reference to Pl. VIII, Fig. 18, shows five spaces between the bundles of long muscles on the dorsal side through which pass the dorso-ventral muscles, nerves, and

[8] Arnold Graf: "Beiträge zur Kenntniss der Exkretionsorgane von Nephelis vulgaris." *Jenaische Zeitschrift für Wissenschaft*, N. F., Bd. xxi, p. 163, 1893.

blood vessels, and it is directly over these or some of them that the pigment collects. I hope to make some further observations on this point by raising the progeny of one leech by themselves until they attain the adult markings.

### *Description of Nephelis Lateralis.*

Since the analysis of Clepsine by Whitman (9) has given the prostomium the value of a somite consisting of one ring, I have followed the notation used by him and have counted the prostomium as ring No. 1 and somite No. 1.

Excepting the clitellum during its active phase, the body is not divided into obvious regions. The oral sucker is not prominent as in some species of Clepsine and the anal sucker is small, exceeding the body but little in width. The male orifice lies normally between rings 36 and 37. The female orifice lies normally between rings 38 and 39 (Pl. VI, Fig. 3).

The first pair of nephridiopores lies between rings 16 and 17 at the posterior edge of the 7th somite. There are four pairs of nephridia anterior to the male orifice, and these differ from the succeeding nephridia by reduction of certain parts. The pores of the first pair of nephridia behind the male pore lie between rings 36 and 37, about midway between the median plane and the margin, and these are followed in regular order, at intervals of five rings, by the remaining pores. The last pores lie between rings 96 and 97, and the whole number of pairs of nephridia is seventeen. The anus is dorsal and lies behind the 104th ring.

The clitellum consists of fifteen rings — from 28 to 42 inclusive. It includes the last four rings of somite X and the first of somite XIII. It is plainly visible only during sexual activity; at other times it can scarcely be distinguished from the adjacent rings. In the active condition it is paler in color and may be swollen so as to become larger than any other part of the body.

[9] "The Metamerism of Clepsine." *Festschrift für Leuckart*, p. 395, 1895.

*Somites.*

The number of rings in the typical somite is five, but this number is reduced at each extremity. Unlike Clepsine, Macrobdella, Hirudo, and some other leeches, Nephelis does not have the first ring of each somite, except in the anal region, marked by especially large sensillae, and the study of a large number of individuals showed the arrangement of sensillae to be constant in this region. The last nephridiopores lie between rings 96 and 97, and 97 is well marked by sensillae (see Pl. VI, Fig. 7). This, then, is the first ring of a somite. The next four rings following have no prominent sensillae, but ring 102 is again strongly marked with them ; 103 is a broad double ring ; 104 is another double ring, the latter half of which bears sensillae. The anus sometimes divides this part of the ring and so comes to be bounded anteriorly by 103, but generally a thin portion of 104 forms the anterior lip of the anus. Rings 102, 103, and the anterior half of 104 make up a pre-anal abbreviated somite, while rings 97 to 101 form a complete post-nephridial somite. Now going forward as far as the first pair of nephridiopores (Fig. 3) the somites may be readily traced by the nephridial openings, and they consist of five rings each. At this point another criterion enables us to determine one complete pre-nephridial somite. The ganglion in each typical somite lies almost wholly in the first ring. If we count five rings forward from the first nephridiopores, we find the first ganglion of the nerve cord lying in this ring, the 12th. The innervation of these five rings also proves that they make up a complete somite. To recapitulate : The somite anterior to the anus is reduced to two and a half, morphologically four, rings ; thence forward to the 12th ring inclusive we find eighteen complete somites, innervated by the eighteen separate ganglia of the nerve cord.

The reduced somites of the head region are innervated from the "brain" and sub-oesophageal ganglia, while the reduced somites of the anal region are innervated from the anal ganglia.

*Head Region.*

The innervation of the rings of the head region shows, as will be demonstrated later, the limits of the reduced somites to be as follows : (Fig. 3) I consists of the prostomium ; II of rings No. 2 and 3 ; III of a single broad ring, No. 4 ; IV of a single ring, No. 5 (this ring lies in the plane of flexion of the body on the oral sucker and is very narrow) ; V consists of three rings, Nos. 6, 7, and 8 ; VI consists also of three rings, Nos. 9, 10, and 11.

*Anal Region.*

In the anal region (Pl. VI, Figs. 3 and 7) the innervation shows the limits as follows : XXV consists of rings 102, 103, and the anterior half of 104. XXVI consists of the posterior half of 104 and 105. 105 is a broad ring which in some individuals shows a tendency to divide into two, sometimes three, rings. It lies in the plane of flexion between the body and the anal sucker. XXVII consists of 106, the last ring of the body and the dorsal area of the sucker. XXVIII to XXXIV consist of the sucker disc. External evidence of this is found in the six radial lines of sensillae on either side of the median plane.

*Summary.*

The number of rings is 106 from prostomium to sucker. The first pair of eyes (Fig. 4) lies in the 2d ring ; the second pair lies wholly in the 4th, while the third pair lies usually between the 4th and 5th rings. The clitellum consists of fifteen rings, Nos. 28 to 42 inclusive, or the 2d to 5th ring of somite X, the ten rings of XI and XII, and the 1st ring of somite XIII. The male orifice lies, usually, between the 36th and 37th rings. The female orifice between the 38th and 39th rings.

The anus opens in the hinder portion of the 104th ring, or between the 104th and 105th rings.

The first nephridiopore lies between the 16th and 17th rings ; the last and 17th nephridiopore lies between the 96th and 97th rings.

The head region consists of the first six somites, comprising the first eleven rings. The first body ganglion lies in the 12th ring, the 1st ring of somite VII.

The 18th and last body ganglion lies in the 97th ring, the 1st ring of somite XXIV.

The body region extends from ring 12 to ring 101, somites VII to XXIV inclusive.

The anal region extends from ring 102 to the disc of the sucker; somites XXV, XXVI, and XXVII.

The sucker contains seven somites, XXVIII to XXXIV.

## Habitat.

Like other leeches, Nephelis keeps its body for the most part in the dark, and must be sought for according to the conditions of the bottom of the pond or stream. In a stony brook or pond beach they may be found adhering to the underside of the stones; on a sand beach unshaded from the sun they bury themselves almost completely in the sand, projecting their heads at short intervals in search of food. Where the overhanging trees have dropped their leaves into the water they will be found on the underside of the leaves. They may be found on the underside of the water-lily leaves, on floating pieces of wood, and between the bark and the wood of rotting, water-logged branches of trees.

They thrive under widely different conditions of water, soil, and temperature, so long as food is obtainable. I have collected them in the Charles River at Cambridge, Mass., during low water in midsummer, when the river was reeking with sewage and the chemical wastes from paper mills, while the temperature was but a few degrees lower than that of the air. Yet, within a stone's throw of the river bank I have collected them quite as readily in a clear spring-water brook, in which the water was so cold that collecting in it was almost painful. The abundant food supply appeared to be the only feature common to the two places.

The character of the bottom of a pond seems to be an indifferent factor, for in the same pond they may be as numer-

ous on a mud bottom as on a sand bottom. This was a matter of surprise to me until I found the explanation. I noticed that I invariably made the best collections on the shore that looks towards the prevailing summer winds ; that is, the shore towards which the surface current flows, bringing with it crustaceans, dead fish, and various other food materials. The windward shore is almost always barren of Nephelis, for the water on that shore is the cool water of the deeper parts and is poor in food for Nephelis. That this food supplying current is the important factor in influencing the distribution of Nephelis is beautifully demonstrated in the small fresh-water ponds near Woods Holl.

These ponds lie in basins scooped out by glacial action, and many of them have no outlet. Some are nearly circular, others are elliptical or long and narrow. The surrounding hills are comparatively high, and the direction of the prevailing wind over the pond is frequently determined by the trend of the lower land or valley near the pond. This exposure to wind varies in different ponds lying near together, and Nephelis are always more abundant on the lee shore. In brooks they are usually more abundant near the mouth of the stream, whether it flows into another stream or into a pond. This is explained in the same way. Food brought down by the brook is more plentiful at that point than at any other.

## Habits, Food, etc.

Nephelis, like Aulostoma, is non-parasitic and differs from the parasitic leeches in many of its habits. It does not readily leave the water like Hirudo or Macrobdella, and in confinement it seldom attempts to leave the aquarium after the first twenty-four hours, if there be plenty of food. It swims freely and rapidly with the same undulating movement that Hirudo employs. In creeping it never brings the anal sucker up to the oral sucker as Clepsine, Hirudo, and Macrobdella, but usually attaches it about halfway between the two in the outstretched body. In common with other leeches, Nephelis has the habit of fixing itself by the anal sucker and then undulating

its body as in swimming. In repose it commonly seeks shelter under a stone, a leaf, a clump of weeds, or in the upper layer of mud or sand at the bottom, exposing only the anterior third of the body. It rests in this position for comparatively long periods and seems, at times, to be sleeping, or at least so sluggish as to require considerable stimulating before it responds. Sometimes it rests curled up in a spiral with its head in the center and attached by its anal sucker. When undisturbed and active it creeps in search of food and stopping now and then it attaches itself by the anal sucker and explores an arc of the circle of which its body is the radius. The head sways from right to left, up and down, while the body is extended gradually to full length ; then the body is shortened and moved through a small angle and the first process is repeated.

When hungry, either at rest or creeping in search of food, Nephelis is quick to perceive its presence ; but while swimming it seems to be less attracted, although it may swim nearly in contact with the foodstuff. My experiments were made on leeches in aquaria, purposely left without food for some days. Leeches fresh from the pond gave practically the same results as leeches that had been kept in confinement for long periods. When the water is about 3 cm. deep an individual at rest on the bottom will perceive a portion of food let down gently overhead almost as soon as it touches the surface ; after a short interval, fifteen or twenty seconds, the leeches lying from 4 to 6 cm. away will give evidence of perception and they will set out to find it. If at another time, when the leeches are bunched together in a mass, the food be placed about 10 cm. away, a minute or a minute and a half may elapse before one shows any sign of awakening and starting in search of the food. Others follow more or less rapidly at intervals. Under these conditions there seems to be some evidence of a sense of direction, but it is vague, if not mere chance. Some, not always the first ones, will start off in the proper direction; others will stray afar ; some will come within 1 cm. of the food and pass on without noticing it ; others will start to swim briskly in irregular paths as if to trace the scent, and when near the food will suddenly settle down, fix the anal sucker,

and explore. They are as likely to explore away from the food as towards it. If, while swimming, any portion of the body touches the food, a leech will often perceive it, stop short, and feed on it.

If a leech is feeding, any other leech that comes in contact with it perceives instantly what the other is doing and rapidly creeps along its body to partake of the meal. I have frequently started up every individual in a bunch of a dozen or more, by gently pushing a morsel to one which projected its head a little beyond the others. The first motion of seizure would be enough to set the whole bunch in commotion. If a bit of food be gently placed on the back of an individual at rest, it will often whirl rapidly about and seize it, though it remains indifferent to another leech creeping over the same place. If, in a clear aquarium containing some hungry Nephelis, the finger be rubbed over the bottom and continued up the side out of the water, the leeches as they creep along the bottom will perceive the scent and follow the trail, even to some distance out of water.

These experiments indicate the same general conditions of perception as Professor Whitman has found in Macrobdella (10).

In the summer time Nephelis lives in the shallow waters of the pond, but in winter it goes down to the deeper parts or into the mud of the edges if there is a good food supply. I have found them in midwinter in seven feet of water when the ice was 25 cm. thick, and in another place in the mud near the edge when the ice was 50 cm. thick, leaving only 8 or 10 cm. of water over the mud. In both cases the individuals were as active as in summer, and some of them laid eggs after being a few weeks in the aquaria. These developed and produced normal individuals which in one instance gave me a supply of small individuals very opportunely.

## Nervous System.

When this work on the nervous system of Nephelis was begun, the chief object in view was to determine the innervation of the somites as a means of elucidating the metamerism of Nephelis.

[10] "The Leeches of Japan." *Quar. Journ. Micr. Soc.*, vol. xxvi, p. 317, 1886.

Professor Whitman was making a study of the nervous system of Clepsine (9) for this purpose, and suggested that the same be done with Nephelis, in order to bring the two genera into comparison. In order that the relations between the two may be clear, I present the following summary of his paper, so far as it bears on this question.

He presents some considerations drawn from embryological evidence to show that the head includes a number of true metameres. "Does it include anything more?"

"In the adult head we find the segments fairly well defined behind the eyes, but how far the metameric division extends into the prae-ocular region remains to be determined. With reference to the origin of the head, we are compelled to take one of two views. The head consists either (1) of a non-metameric lobe plus a number of metameres originally belonging to the trunk, or (2) of such metameres only, the non-metameric head element of the ancestral form having been lost or incorporated in the first metamere."

Each body neuromere in Clepsine, disregarding the longitudinal nerve cords which fuse regularly at the level of each metameric center, comprises three pairs of nerves and six ganglionic masses, each mass being contained in its own capsule. Two of these are always ventral and median, the remaining four are arranged in pairs, two on either side above the nerve roots. The sub-oesophageal ganglia readily show their metameric origin; the ventral capsules of the body neuromeres persist, arranged in a median row with only the two anterior capsules crowded into bilateral positions. The corresponding lateral capsules are readily identified in the 6th, 5th, and 4th segments, while the others in the 3d and 2d have been crowded out of the places they would naturally occupy. The nerves from this region are also identified as containing the elements of the single neuromere. VI, V, and IV have three roots each; III shows only two roots, and II issues as a single root, which soon divides into two branches. Sections show very plainly the presence of five nerve roots, each with its pair of median nerve cells. Thus the evidence is conclusive that the sub-oesophageal region consists of five metameres (II to VI).

"The surprising thing is that we have left what seems to be the exact equivalent of a trunk neuromere; *one pair of nerves* (1) *and six ganglionic sacs,* of which two are median and four are lateral. Whether there is a pair of 'median nerve cells' connected with this part of the nervous system, I cannot say. I have not found them, but my search has not been exhaustive. The equivalence in other respects is so complete that there seems to be no escape from the conclusion that *the ganglionic centers of the ventral cord are simple repetitions, element for element, of the 'brain.'* The nervous system is made up of segments of equal morphological value throughout. It must be regarded then either as a series of 'brains' or as a series of ventral neuromeres, one or more of which have been carried secondarily to the dorsal side, and which here take the place of a brain that has been lost or confounded with the metameric system. That a portion of neuromere II has suffered transportation from the ventral to the dorsal side is certain; but the development of the supra-oesophageal system does not permit us to believe that neuromere I was ever post-oral in position. Allowing that it represents genetically the annelid brain, as it certainly seems to do, the ventral cord must be regarded as a chain of brains. The *dorsal* position of the brain signifies nothing more than that the anterior end of the double nerve cord has been bent upward from its prae-oral and ventral position and slipped backward over the oesophagus."

In the caudal region, although the concentration is quite as great as in the head region, the elements of the neuromeres are plainly resolvable. Each neuromere is complete in the number of capsules and the nerve roots, which, however, are here reduced to two. The whole nerve chain is divisible into three portions: the head with six neuromeres, the trunk with twenty-one, and the caudal disc with seven, making a total of thirty-four neuromeres. Referring to Pl. I (Pl. IV here), the innervation of a typical body somite is made clear. We find the nerve divided into *three* distinct parts which we may designate as anterior, middle, and posterior nerve, respectively. "A glance will make clear one very interesting feature in the distribution of these nerves. They *innervate three successive rings,*

*the first and second of their own segment, and the third of the preceding segment.* The distribution is thus triannulate and dimeric."

Passing to the head region, we find a number of interesting modifications of the plan found in the body somites. Nerve VI has three parts, "but they are no longer the precise equivalents of 'anterior,' 'middle,' and 'posterior' nerves. What before appeared as the dorsal branch of the posterior nerve now appears as the middle nerve, supplying the same sense organs as before and, in addition, the inner lateral sense organ of segment V. The third nerve has no dorsal branch except the short one to the outer lateral sense organ. It has two main branches, however, one of which takes the place of the 'middle' nerve, the other that of the 'posterior' nerve. The first nerve alone remains the unchanged 'anterior' nerve. The branch running to the inner lateral sense organ (*i.l.*) of segment V belongs, according to what we saw in typical segments, not to segment VI, but to segment V.

"In segment V we find three nerves, but their composition and distribution depart still further from the typical arrangement. This nerve, as shown in Pl. I [Pl. IV here], gives off a number of motor branches, and then passes to the outer lateral and marginal sense organs and the labial organs of four rings (8–12). It innervates then the first and second rings of its own segment, and two rings (9–10) of segment IV. It corresponds then to the 'middle' nerve in the trunk region, but contains also fibers belonging to three other nerves, namely, the 'posterior' nerve of the preceding segment, and the 'anterior' and 'posterior' of its own segment. Just above and a little in advance of this root appears another quite strong nerve, which rises and passes forward over the lateral angle of the supra-oesophageal ganglia. This nerve divides just in front of the head ganglia, sending one branch to the inner lateral organ of segment IV, and the other to the median organs of segments IV and V. This nerve then corresponds to the dorsal sensory branch of a 'posterior' nerve, and includes so far as it goes the fibers of two such branches, for segments IV and V.

"In segment IV we find only two nerves, one small motor, corresponding to the 'anterior' nerve, and one large nerve

which, after giving off several motor nerves, runs to the labial sense organs of three rings (6–8) and to the outer lateral organ of ring 8 in its own segment. This nerve corresponds in the main to a 'middle' nerve. The sensory 'dorsal branch' of the 'posterior' nerve of this segment, as we have seen, is united with the corresponding nerve of segment V.

"In segment III we find only two nerves, corresponding with the two seen in segment IV. Where is the sensory 'dorsal branch'? On examining nerve II, we find it contains the missing nerve united with the corresponding nerve of segment II. Nerve II supplies not only the rudimentary eyes (median sense organs) of its own segment, but also the pair of large eyes and the inner lateral organ of segment III. One of its two main branches supplies the outer lateral organ and the labial organs of segment II.

"Nerve I innervates the median, the inner lateral, and labial sense organs of the most anterior division of the head."

This species shows also very plainly that some of the metameric sense organs acquire eye-like properties in the head region which gradually increase towards the anterior somites. "In no other species hitherto described do we find the sensillae passing by such gradations into the eyes. *The serial homology of these organs with the eyes is then a fact demonstrated not only by the embryonic development, but also by the structural gradations in the adult animal.*"

In the concluding portion Professor Whitman reviews the evidence derived from the innervation of the head region and says: "The morphological equivalence of segment I with the following segments is evident to a degree that is really astonishing. It makes no departure from the typical trunk segment which is not led up to through gradations represented in the segments immediately following it."

## The Nervous System of Nephelis.

The nervous system of Nephelis may for convenience be divided into two parts: that portion which responds to external stimuli and coördinates the muscles of locomotion, the central

nervous system; and that portion intimately connected with the control of the organs of internal life which I shall call the sympathetic system. These two parts differ widely in certain characteristics of structure as well as of function. The central nervous system is strongly metameric throughout its length. Its cells are relatively larger and are referable to the unipolar and bipolar types for the most part. The fibers of these cells always tend to run in bundles and never to form plexuses. The sympathetic, on the other hand, is free from any discoverable trace of metamerism; its cells are small and frequently multipolar, and the fibers always tend to form plexuses (Pl. VIII, Fig. 19).

*The Central Nervous System.*

The entire ganglionic chain in Nephelis, as in other leeches, is contained in the ventral blood sinus, which, according to Bourne and others, is one of the vestiges of the original coelomic cavity. This sinus runs directly under the alimentary canal and is readily distinguished by its dark pigmentation and the swellings within which lie the ganglia.

The anterior end of the chain, called the sub-oesophageal ganglia and the brain, consists of a mass of neuromeres more or less completely fused together, and forming a collar about the oesophagus. The posterior end, called the "anal ganglia," consists likewise of a number of neuromeres more or less completely fused. Between these terminal portions lie eighteen neuromeres joined each to the next by two connectives. Between these, and dorsal to the axis of the chain, lies a small bundle of fibers known as the median nerve, or Faivre's nerve. These connectives are longest in the mid-body region and decrease in length towards either end of the body, becoming almost nil in the most fused parts at both extremities. Within each connective lies a "colossal axial" cell, the nucleus lying about midway between the neuromeres, as has been described for other leeches.

At the points of junction between the connectives and the neuromeres the fibers of the connectives do not separate into small bundles as they do in Hirudo and Macrobdella, but each continues into the body of the neuromere as a single bundle.

### *A Typical Neuromere.*

In order to analyze the "brain" and the "anal ganglia" it is necessary to know the component parts of a typical neuromere and to grasp their relations to each other under normal conditions.

The general shape of a ganglion is that of a flattened ellipsoid, the long axis of which is parallel to that of the body; the ventral surface being slightly more convex than the dorsal (Pl. VI, Fig. 9). Each ganglion gives rise to two nerves on each side which leave the ganglion and proceed for a short distance in a horizontal plane, and then branching, go to the dorsal or ventral side.

The anterior nerve, however, is not a single nerve. It results from the fusion of a ventral and a dorsal root, the fusion taking place almost immediately after their departure from the body of the ganglion (Pl. VI, Fig. 9). This fact enables us to homologize the two lateral nerves of Nephelis with the three of Clepsine as follows: I and II in Clepsine are represented by I in Nephelis. III in Clepsine is II in Nephelis. This homology is also shown by the correspondence of the areas innervated by I in Nephelis and I and II in Clepsine (Pll. IV and V). I have not been able to find evidence of the similar origin of the anterior nerve in Hirudo or Macrobdella, and this fact suggests that Nephelis is an intermediate form between the Clepsinidae and the five-ring leeches.

Between each pair of the lateral nerves, and near the ganglion, lies a bipolar cell, the principal prolongations of which pass outward along the trunks of the lateral nerves for a short distance and then fuse with them so as to be indistinguishable from them. This cell is found in other Hirudinea and has been called from its discoverer "Leydig's cell." Its presence throughout the entire ganglion chain, its variation under different conditions, and its possible relations to other extraganglionic cells are of sufficient interest to demand for it separate consideration.

The nerve cells of the ganglion, with the exception of "Leydig's cell," are gathered into six groups or clusters lying

outside of the central fibrous portion in capsules as in the other Hirudinea. Pl. VI, Fig 9, shows the general arrangement. Two clusters lie on the ventral surface in the median line (one anterior and the other posterior) and two clusters on each lateral face. The anterior lateral clusters are anterior to the anterior nerves, and the posterior lateral clusters lie between the two nerves. These lateral clusters rise slightly above the dorsal surface of the body of the ganglion, and their posterior edges are notched by the lateral nerves as they pass out from the ganglion (Pl. VI, Fig. 12). The number six is constant in the whole chain and the position is also constant except in the supra-oesophageal ganglia or "brain." In the "anal ganglia" the lateral clusters tend to become dorsal towards the posterior portion owing to compression, but they are perfectly recognizable and referable to their proper neuromeres (Pl. VI, Figs. 14 and 15).

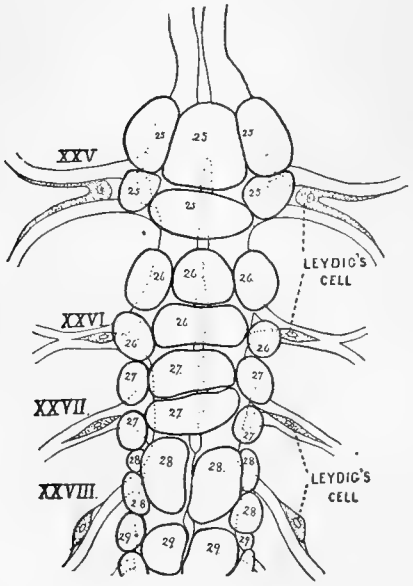
### *Leydig's Cell.*

Lying between the two nerve trunks of either side of the ganglion, and nearly in contact with the posterior lateral capsule, lies a large bipolar cell whose prolongations follow along the lateral nerves as they pass outward, and finally fuse with them (Pl. VI, Fig. 9). This cell was described first by Leydig in *Hirudo medicinalis;* it was called "Leydig's cell" by Hermann (11), and is found in all the Gnathobdellidae in the same relation to the ganglion. I have not been able to find any trace of fibers going to the ganglion; so far as I have traced them they all pass outward along the nerves. This cell, the significance of which is as yet entirely unknown, is constant throughout the central nervous system. I have found it in every neuromere of the body. In the first four neuromeres it lies upon the fused nerves at some distance anterior to the "brain" (Pl. V, Fig. 2). In the fifth neuromere (Pl. VI, Fig. 11) the cell is found at the angle formed by the separation of the hitherto fused portions of the nerve trunks. In the sixth neuromere, the last nerve of the sub-oesophageal mass, the cell

[11] Ernst Hermann: Das Central Nervensystem von Hirudo medicinalis. München. 1875.

lies much closer to the mass and exhibits more of the characters of the normal cells found in the body ganglia.

In the "anal ganglia" (Pl. VI, Figs. 14 and 15, and Fig. 1 in the text), the first neuromere, XXV, the cell is normal. In the second, XXVI, the two nerves partly fuse at a little distance from the margin, and continue thus for a short distance, when they become fully separate. Within this region of partial fusion "Leydig's cell" is found lying between the two trunks compressed and changed into a spindle-shaped cell; the prolongations extending median and lateral as in the cells of the first four neuromeres. In the third "anal" neuromere, XXVII, the cell appears in the same relative position, but is more compressed and elongated. Good histological preparations of these cells show that the size and structure of the nucleus and the nucleoli are identical with those of a cell from a mid-body region.




Text-fig. 1. — The four anterior neuromeres of the "anal ganglia" seen from the ventral side, showing the stages of compression of "Leydig's cell" till it appears outside of the fused trunk in XXVIII (1st anal in Clepsine) and the succeeding nerves. (From a camera drawing of a nitric acid preparation. The details of the "Leydig's cell" were supplied from sections.)

The fourth, XXVIII, and succeeding neuromeres, XXIX to XXXIV, innervate the sucker. The fusion here is as complete as in the first four neuromeres, and the "Leydig's cell" has been pushed out, in the more complete fusion of the nerve trunks, until it lies completely outside of and upon the nerve, about midway from the anal ganglion to the edge of the sucker; the prolongations extending, as before, median and lateral. In these most posterior neuromeres the size and

structure of the characteristic features of the cell remain unchanged from those of the normal mid-body cell.

### *Median Nerve Cells.*

Within the fibrous body portion of the normal ganglion, near the median plane, lie two "median nerve cells," one slightly anterior, the other posterior, to the center. They are found in all the Hirudinea and have been described by several authors. Retzius (12) and Biedermann (13) show them in their figures of Hirudo obtained by methylen blue, and they continue to appear forward in the sub-oesophageal ganglia. In my analysis of the "brain" I shall speak of these in detail. I have also found them in the anterior neuromeres of the "anal ganglia," but I am not able to say whether they are present in the posterior neuromeres or not.

### *The Fibrous Portion.*

The fibrous part of the ganglion occupies the axial portion and, macroscopically, appears to consist of thickenings of the two connectives that afterwards fuse. It is perforated by two small holes which lie close together on either side of the median plane at the level of the anterior nerves. These perforations persist in the fused portions of the nerve chain and afford good evidence of the fusion of originally separate neuromeres.

According to Biedermann (*l. c.*) and Retzius (*l. c.*), this fibrous part is made up of fibers from three different sources : (1) from the connectives, part of which continue through the ganglion ; (2) the efferent fibers from the neurones, which fill the six capsules of the ganglion ; and (3) the afferent fibers, which are the central termini of neurones whose trophic centers lie outside of the ganglia.

The first two sources are readily demonstrated, but Retzius failed to find the source of all the fibers in the third set. His

[12] G. Retzius : Biologische Untersuchungen. Neue Folge, 2. Stockholm. 1890.

[13] W. Biedermann : "Ueber den Ursprung und die Endigungsweise der Nerven in den Ganglien wirbelloser Thiere." *Jenaische Zeitschrift für Naturwiss.*, Bd. xxv, 1891.

figures are very clear and show the structure of the fibrous portion of the ganglion with great detail.

He traces out the course of the axis cylinders from the cells of each capsule, and of those fibers whose trophic centers lie outside of the ganglion. He separates these fibers into six groups, five of which come into the ganglion by way of the connectives and one by way of the lateral nerves. This last group, the sixth of Retzius, is of peculiar interest, because while Retzius was drawn by his examination farther and farther from the ganglion to search for the cell-bodies of this class of fibers, until he reached the epidermis, he did not succeed in finding them.

He says (N. F., ii, p. 21): "Was stellen nun diese Fasern dar? Sie sind offenbar Nervenfasern, welche peripherisch verlaufen. Wo sind aber ihre Ganglienzellen? Da ich bei den Crustaceen ähnliche, durch die peripheren Nervenzweige aus den Ganglien des Bauchstrangs, austretende Nervenfasern mit grossen in den Ganglien befindlichen Ganglienzellen in Verbindung gefunden hatte, so schien es mir auch bei den Hirudineen möglich zu sein, dass die fraglichen Fasern von intraganglionären Zellen entspringen könnten. Es erwies aber durch zahlreiche Versuche, dass dieses nicht der Fall war; keine Ganglienzellen konnten mit ihnen in Verbindung angetroffen werden.

"Die fraglichen Fasern treten offenbar von der Peripherie her in die Ganglien hinein, um hinter der geschilderten Verästelung sich in ihre Punktsubstanz aufzulösen.

"Der von Hermann u. a. gemachte Befund grosser Ganglienzellen im Verlauf der peripheren Nervenzweige erklärt aber in sehr plausibler Weise ihre morphologische Bedeutung; sie müssen eine Art von Nebenfortsätzen dieser peripheren Ganglienzellen darstellen, welche durch sie die contactartige Verbindung mit den Elementen der Ganglien, d. h. dem centralen Nervensystem, aufrecht erhalten. Ich versuchte nun, durch Methylenblaulösung die fraglichen Ganglienzellen und ihre Fortzätze zu färben, aber der Pigmentreichthum und die Scheidenbildungen verhinderten leider bei Aulastoma und Hirudo die endgültige Lösung dieses interessanten Problems."

I believe that I have found the source of these fibers in the bipolar cells that lie in the intermuscular nerve ring, the description of which will be given later.

## Innervation of a Body Metamere.

As I have said before, the ganglion lies in the first ring of the somite and the two lateral nerves pass out, for a little distance, in a horizontal plane and at right angles to the long axis of the body. Then they divide into dorsal and ventral branches, and again divide and subdivide to innervate the various organs, as described in detail below. The first and most striking fact is that the distribution is morphologically identical with that of Clepsine, and the second that it confirms Professor Whitman's explanation of the derivation of the five-ring metamere from a three-ring type: *e.g.*, Clepsine.

A glance at Pl. V, metamere VIII, will show that the anterior nerve innervates the 4th and 5th annuli of the preceding metamere on the ventral side and the extreme lateral sensillae of the 1st annulus of its own metamere, and sends fibers to the intermuscular nerve ring in the 5th annulus. The posterior nerve sends one ventral branch to the sense organs on the ventral side of the 3d annulus and two ventral branches to the intermuscular nerve ring in the 2d annulus, one of which by subdivision makes two connections with the nerve ring. The principal branch of the posterior nerve is dorsal, and this branch innervates, first, the few dorsal sensillae on the 4th annulus of the preceding metamere; second, the dorsal side of the nerve ring in the 5th annulus; third, the large sensillae in the 1st ring of its somite; fourth, the dorsal side of the nerve ring in the 2d annulus; and fifth, a few dorsal sensillae in the 3d annulus. A comparison now with Professor Whitman's work on Clepsine will show how completely identical the distribution is (Pll. IV and V). The 4th and 5th annuli are morphologically the 3d annulus of Clepsine; they are innervated by ventral portions of the anterior nerve, as in Clepsine. The branch of the anterior nerve in Nephelis that represents the middle nerve in Clepsine innervates exactly

the corresponding area in Nephelis, the ventral side of the 1st annulus together with the outer lateral sensillae. The posterior nerve in Nephelis, as in Clepsine, is the principal sensory nerve, innervates dorsal sensillae in all five rings, and ventral organs in annuli 2 and 3 (Clepsine 2).

Remembering, then, that annuli 4 and 5 represent annulus 3 in Clepsine, that 2 and 3 represent annulus 2 in Clepsine, together with the homology of the nerves, the anterior nerve of Nephelis representing the anterior and middle nerves of Clepsine, we may use Professor Whitman's words (9, p. 388) to describe the distribution of the nerves of Clepsine for Nephelis as well: "They innervate three successive rings, the 1st and 2d of their own segment and the 3d of the preceding segment. The distribution is thus triannulate and dimeric."

## The Innervation of the Terminal Somites.

We are now prepared to understand the modifications of the plan of innervation found in a body somite as found in the somites innervated by the more or less completely fused terminal neuromeres in the head region, somites I to V, and those in the anal region, somites XXV to XXXIV. These I shall call terminal somites for convenience. Of these two groups, those of the anal region present less departure from the normal and hence will be described first.

### *The Anal Region.*

As I have stated briefly elsewhere, the posterior portion of the nerve chain is sometimes called the anal ganglia. As Whitman and others have shown in other leeches, so in Nephelis the nerve chain in this region consists of neuromeres more or less fused together but retaining their fundamental characteristics to such an extent that they can be easily identified. Pl. VI, Figs. 14 and 15, show the dorsal and ventral views. The number of neuromeres is ten, — the three anterior being less modified by the fusion than the remaining seven. Koehler (14)

[14] R. Koehler: Recherches sur la structure du système nerveux de la Nephelis. 8°. Nancy. 1882.

is quoted by François (15) as assigning nine neuromeres to the anal ganglia. The 1st anal neuromere, XXV, innervates the first of the posterior terminal somites (Pl. V, Fig. 7), consisting of annulus 102, the somewhat double annulus 103, and the anterior half of 104. The 2d anal, XXVI, innervates the anus-bearing somite, the posterior half of annulus 104, and the broad annulus 105, which often shows traces of doubling. The 3d anal, XXVII, innervates the last somite preceding the sucker, which consists of annulus 106 and the "acetabulum," the area lying between the last annulus and the sucker. This area lies in the plane of flexion of the body on the sucker and has lost all trace of annulation. The succeeding seven neuromeres innervate the sucker. The 4th anal, XXVIII, innervates the anterior part of the sucker, immediately on either side of the median plane. The last anal, XXXIV, innervates the posterior part of the sucker in the same way, while the intermediate neuromeres supply the rest of the sucker radially between these parts. The XXVth or 1st anal somite resembles in every particular a normal body somite. The connectives to the XXVIth are very short and broad, and they become shorter and more nearly uniform in breadth with the body of the ganglion as we continue backward. They preserve their characters as connectives, however, as is shown by the oblong slits in the central fibrous portion, until the XXXIId neuromere is reached, when the slits cease. The nerves of the XXVIth and succeeding neuromeres are single, and the details of their fusion have already been described under the "Leydig's cell." The arrangement of the capsules is interesting, for they help to give evidence of the relations between Clepsine and Nephelis (Fig. 1 in the text). The ventral capsules of a normal neuromere lie one in front of the other, so that the line separating them is transverse. This obtains in XXV, XXVI, and XXVII, the first three neuromeres of the "anal ganglia." But in XXVIII and the succeeding neuromeres the ventral capsules lie side by side, the line separating them being longitudinal.

[15] Ph. François: Contribution à l'étude du système nerveux central des hirudinées. Poitiers. 1885.

In discussing the anal region of Clepsine, in which the same condition obtains, Whitman says (9, p. 388): "This arrangement, evidently one of mechanical adjustment necessitated by the shortening and crowding of the segments, prevails throughout the caudal region with the exception of the first segment (XXVIII) in which the sacs are placed one behind the other as in typical trunk segments."

Again, in his description of *Clepsine plana* (16, p. 413), he says: "Reduction, as I have before pointed out, seems to have begun at both extremities, and to have advanced from these points towards the middle of the body. Its advance shows how far a form has departed from the ancestral condition of uniform somites. It is here that we discover a very important guide to the systematic rank and relationship of different forms." These seven neuromeres, then, correspond to the entire anal ganglia of Clepsine, as is shown by the degree of fusion in the lateral nerves and in the arrangement of the ventral capsules. The process of reduction in the anal region has gone on further in Nephelis by three metameres than in Clepsine, while in the head region the number remains the same in both forms.

### *The Head Region.*

In order to make an analysis of the terminal somites of the head region we must keep in mind that the external criteria of a neuromere are six capsules, two being ventral, two pairs of nerves, and a pair of "Leydig's cells." Beginning at the posterior end and working forwards we shall have little, if any, difficulty in finding six neuromeres.

The nerves of the last neuromeres, VI (Pl. VI, Figs. 10–13), arise as single trunks, but divide very near the body of the ganglion, and in the angle of separation of each trunk lies a "Leydig's cell." Two pairs of lateral capsules, 6.6., separated from the others, are easily identified as belonging to this nerve, so that with the two end capsules of the two ventral series we find all the elements of the typical body neuromeres. The next nerve, V, arises as a single trunk and proceeds forwards as

[16] "Description of Clepsine plana." *Journ. of Morph.*, vol. iv, 1891.

such until it passes the collar, when it divides into a ventral and dorsal branch, and at this point of separation, as in VI, lies a "Leydig's cell" (Pl. VII, Fig. 16). Two pairs of lateral capsules lie well separated from the others, just anterior to those belonging to VI, and two more, 5.5., of the ventral series furnish the elements of this neuromere, V. The next nerve, IV, arises as a single trunk, proceeds for a much longer distance as a single trunk, sending off to the 5th annulus a dorsal branch which quickly divides (Pl. V). This annulus is a very narrow ring lying in the plane of flexion of the oral sucker and the body. The "Leydig's cell" of this neuromere lies completely outside of the nerve trunk, just as it does in XXVIII or the 4th anal neuromere, and sends one fiber forward and one backward (Pl. VII, Fig. 16). The two pairs of lateral capsules belonging to the neuromere lie just anterior to those of neuromere V close to the angle made by the collar. The third capsule of the cluster at this point, lying close to, and anterior to, these two, belongs to the next neuromere, III (Pl. VI, Figs. 11 and 13). These two lateral pairs of capsules, together with two, 4.4., of the ventral capsules, complete the elements of neuromere IV. The next nerve, III, arises just anterior to IV and proceeds in much the same manner, dividing near annulus 5 into dorsal and ventral branches. The "Leydig's cell" lies alongside the trunk, as in IV (Pl. VII, Fig. 16). The lateral capsules belonging to this neuromere show the same peculiarity that Whitman found in Clepsine and that I have seen in Macrobdella,—one pair lying close to the capsules belonging to neuromere IV, while the other pair lies close to the capsules of II, being separated by a wide space. The two ventral capsules, 3.3., complete the elements of this neuromere (Pl. VI, Figs. 11 and 13).

The next nerve trunk arises from the collar as a single large trunk and proceeds some little distance before it shows evidence of separation, and just after separating a "Leydig's cell" appears on each trunk as in III and IV (Pl. VII, Fig. 16). We have here, then, nerves II and I as their distribution also shows. The lateral capsules of II are situated on the posterior side of the collar, while the most anterior, 2.2., of the ventral capsules

complete the elements of this neuromere. The capsular elements of neuromere I differ from all the others, in that the whole six are carried on the dorsal part of the collar (Pl. VI, Figs. 11 and 13). Excepting the position of the ventral capsules, the supra-oesophageal ganglion does not differ from the typical neuromere, and the argument made by Whitman (9) for Clepsine applies with equal force to Nephelis. Not only do these nerve trunks, "Leydig's cells" and capsules, show by their analysis the presence of six, and only six, neuromeres in the head region, but the distribution in the peripheral parts confirms it and sets the limits to the terminal somites in the most conclusive manner.

The gold chloride stain was peculiarly valuable in this work, and gave me sections with which it was only a question of patience to follow out the well-defined nerve branches to their peripheral parts. The fibers stand out distinct in form and color, not to be confused with any other element in the head. The spherical cysts of a parasitic nematode often furnished excellent data for the perfect superposition of the drawings of a series of sections and made it possible to follow out every fiber represented in my drawings through its subdivisions to the sense organs.

Beginning, as before, at the 11th annulus (Pl. V, Fig. 2) I find the distribution from behind forward as follows: the 11th annulus contains an intermuscular nerve ring, and receives its innervation from the succeeding neuromere, VII. The posterior trunk nerve of VI sends a ventral branch to the 10th annulus and a dorsal branch which innervates dorsal sensillae on the 10th and 9th annuli, as well as sending a branch forward to the intermuscular nerve ring of annulus 8. The anterior branch is wholly ventral and lateral, innervating the intermuscular nerve ring in the 8th annulus and a few ventral sensillae. The 9th, 10th, and 11th annuli, therefore, make up metamere VI, the innervation of which is strictly comparable to that of a body metamere, being dimeric and triannulate. The most striking departure from the five-ring metamere lies in the absence of the intermuscular nerve ring from annulus 10, morphologically the 2d annulus of the

body metamere. Proceeding forwards, the 8th annulus has an intermuscular nerve ring, innervated as has just been described from the succeeding metamere, VI.

The inner, or median, branch of nerve V corresponds to the anterior lateral nerve of a body somite and innervates a few ventral sensillae on the 7th annulus, the outer lateral sensillae of annulus 6, the ventral portion of the intermuscular nerve ring in annulus 5, and thence passing forwards innervates the labial sense organs on the ventral margin of the oral sucker. The outer branch, corresponding to the posterior lateral nerve, rises sharply to the dorsal side (Pl. VII, Fig. 16), innervates the sensillae in the 7th and 6th annuli, and sends a branch to the intermuscular nerve ring in the 5th annulus. The 6th, 7th, and 8th annuli, then, form metamere V, and again we find the dimeric and triannulate distribution found in Clepsine and in the normal body metamere of Nephelis. From this metamere forward the distribution is simpler but readily referable to the body metamere. Annulus 5 is, as has been described, very narrow and situated in the plane of flexion, yet it represents metamere IV, for it contains an intermuscular nerve ring innervated from the succeeding somite, and nerve IV gives off a dorsal branch which, quickly dividing, innervates dorsal sensillae and the third pair of eyes in this annulus, while the ventral branch goes forward to innervate some of the lateral labial sense organs. The persistence of this annulus in the plane of flexion is a striking instance of the stability of the 1st annulus of the metamere. Reduced, by its position, to the narrowest annulus in the animal, so narrow that the eye belonging to it has been forced partly outside of it into the broad 4th annulus, it retains not only the characteristic features of the 1st annulus, but also the intermuscular nerve ring belonging to the posterior annulus of the normal body metamere. Annulus 4 is broad and bears two rows of large sensillae on its dorsal surface. It represents metamere III. Nerve III divides as it enters the annulus, sending off a dorsal branch, which soon divides, one branch going to the sensillae of the annulus, the other innervating the second pair of eyes. The ventral branch goes to labial organs on the dorso-lateral margin.

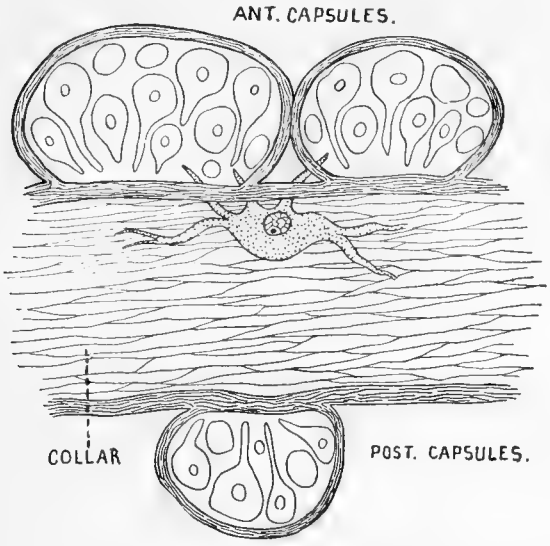
I have found evidences of the intermuscular nerve ring in this annulus, but I have not been able to trace them completely enough to describe the relations which the ring bears to the other parts of the nervous system.

The annuli lying in front of annulus 4 are incomplete, as shown in Pl. VI, Figs. 5 and 6. Annulus 3 is well marked off on the dorsal side, and the groove separating it from 2 is clear and sharp in outline, while 1 is separated from 2 by a partial groove extending about two-thirds of the way across the dorsal surface.

Nerve II innervates the numerous, large, dorsal sensillae of annulus 2, a few small ones on annulus 3, and the large first pair of eyes in annulus 2. The ventral branch of this nerve is reduced to a small branch that traverses the long axis of the first eye and proceeds to a few of the dorsal labial organs. Annuli 3 and 2, therefore, make up metamere II, and the nerves of this metamere, like those described, innervate the preceding metamere. Nerve I innervates annulus 1, supplying the numerous large sensillae and the numerous mid-dorsal labial organs. This fact raises the prostomium to the rank of a metamere, and it must be counted as one. It has been customary to disregard this reduced portion in numbering the metameres and annuli, but hereafter it must be reckoned in the count of metameres and annuli, as Whitman has done in Clepsine (*l. c.*).

Thus far the external features of the sub-oesophageal ganglia and the "brain" and the distribution of the nerves have been analyzed with concordant results; there remains an internal factor that adds still further proof for Professor Whitman's proposition. In Nephelis, as in Clepsine and other Hirudinea, each body neuromere contains, as I have said, two "median nerve cells." In Nephelis, as in Clepsine, they are found in the sub-oesophageal portion, but arranged numerically, four pairs appearing instead of five, as Whitman finds in Clepsine. Careful examination of excellent sections reveals further that in each side of the collar, near the capsules, 2.2., ascribed to metamere II is a "median nerve cell" somewhat irregularly compressed. The volume of the cell is still large, and the

nucleus has the same size and characteristics of the typical "median nerve cell." Still further dorsally, lying between the capsules ascribed to metamere I, I find the pair of remaining "median nerve cells" here compressed into a spindle




TEXT-FIG. 2. — The "median" cell in neuromere I as seen in a horizontal section through the dorsal part of the collar. The section passes through the capsules, 1.1.1., of Fig. 13, Pl. VI. Camera outlines. $\frac{1}{12}$ immersion oc. 3. Reduced one-half.

form (Fig. 2 in the text). This discovery enables us now to say, without reserve, that every element recognized in the body neuromere is found in the supra-oesophageal ganglia, and, therefore, that the supra-oesophageal ganglion or "brain" is homologous with a body neuromere.

## THE INTERMUSCULAR NERVE RING.

Intimately connected with the central nervous system and probably closely related to it in origin is a remarkable peripheral system of nerves hitherto, so far as I have been able to learn, wholly unknown and unsuspected by all investigators who have worked especially on the details of the nervous system of leeches. For the discovery, I must again thank the gold-chloride method of staining, for the only elements of it

that show in control preparations are the large bipolar cells, and these are constant whatever method is employed.

Traces of this system are found in the most anterior portions of the head in the form of large bipolar cells, whose connections I have not yet determined. The 1st ring occurs in metamere IV, annulus 5; the 2d in metamere V, annulus 8; the 3d in metamere VI, annulus 11, the last annulus of the head region. From this point onward two rings are found in each full metamere, in the 2d and 5th annuli respectively. I have not found the ring behind metamere XXIV, or any well-defined traces of it.

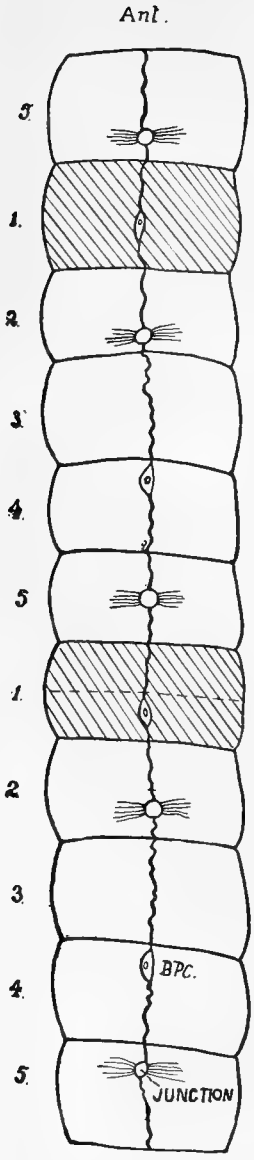
Beginning with metamere VII, I find eight bipolar cells connecting each ring with the succeeding one, as shown in Pl. V and as will be described below. Each ring receives fibers from, and sends fibers to, the central nervous system, and fibers from the ring run to sensillae and muscles.

Figure 18, Pl. VII, is a diagram of one-half of a transection made in the mid-body region, designed to show the arrangement and relations of this nerve ring. It is a projection constructed from camera drawings of the nervous elements in annuli 5 and 1 of adjoining somites upon a single plane, viewed from anterior to posterior. The anterior nerve (compare Pl. V, Fig. 2) runs laterally in the 1st annulus, sending forward two ventral branches, between the 3d and 4th, and the 4th and 5th bundles of long muscles, one to the nerve ring in the 5th annulus, the other, the cut end of which is shown in this figure, to a few ventral sensillae in the 4th annulus. Passing out to the edge, it innervates three large sensillae in annulus 1, as shown. The posterior nerve rises dorsally and innervates three large sensillae on the dorsal surface of annulus 1, and sends forward a branch which goes to the nerve ring between the 4th and 5th bundles of dorsal long muscles and on to a few dorsal sensillae in the 4th annulus. The nerve ring here represented lies in the 5th annulus. It consists of a complete ring of fibers which lie between the layers of long muscles and the circular-oblique muscle layers, whence the name I have given to it. Around this ring at definite points are ten groups of bipolar cells, six

on the dorsal side, four on the ventral side. These cells resemble in size and character of nucleus the "Leydig's cell" of the body neuromere. They all send fibers in both directions into the ring, but I have been so far unable to follow them to their terminations. The groups differ in characteristic features, and for the purpose of description I have named them as shown in the figure. They are constant in their position and character throughout the leech, and this I determined by comparing the six rings in three successive mid-body metameres minutely, detail with detail, by the method described in the early part of this paper.

The inner dorsal group (*in. d. bpc.*) consists of one bipolar cell with a short pedicel, lying in the 3d muscle bundle almost sessile on the ring. The outer dorsal group (*o. d. bpc.*) consists of a cell with a very long pedicel, lying in the 4th muscle bundle. The lateral dorsal group (*lat. d. bpc.*) consists of six or eight cells, four to six of which are small, lying in the outer or 6th muscle bundle.

The outer ventral group (*o. v. bpc.*) consists of one cell with a short pedicel, lying in the 5th muscle bundle, and like the inner ventral group, very near the point of junction with the nerve trunk from the central system. The inner ventral group (*in. v. bpc.*) consists of two or three cells with pedicels of medium length, lying near the edge of the 4th muscle bundle. The connection with the central system at this point is different from the others. The trunk divides just before reaching the ring, and as I have traced fibers from this inner ventral group into that branch nearest the group, it is evident that it carries fibers from these cells to the ganglion, the sensory fibers, while the other branch of the nerve trunk carries motor fibers from the ganglion into the ring. If my data as just given are sufficient, then we have the same morphological conditions that obtain in the spinal ganglion of vertebrates. The one piece of evidence wanting is the termination of the other pole of the cell. These cells are without doubt those which Retzius (*l. c.*) sought, as quoted above (page 42), to find in the epidermis. These cells, together, possibly, with the outer ventral cell, answer the conditions called for by Retzius for




Text-fig. 3. — A very narrow tangential section showing the long bipolar cells running from junction to junction through two metameres and five rings. (Camera drawing from a gold chloride preparation.)

those central endings which enter by the anterior nerve. I have not been able to determine which of the dorsal cells send in fibers by way of the posterior nerve. The ring itself, as shown in the gold-chloride preparations, consists of numerous fine fibers, which come in part from the bipolar cells, in part from the central system, and in part from the diffuse sensillae in the epidermis. It gives off fibrillae which, ramifying the bundles of long muscles, innervate the cells.

As I have said above, these rings are connected, one with another, by long bipolar cells lying between the same muscle layers, as shown in Pl. V. These connecting cells form, when taken together, eight longitudinal paths, reaching, according to my present investigations, from metamere VII through the body region to metamere XXV. I have no doubt that they may be found in the regions of the terminal somites, but I have not yet been able to do so.

These long connective cells join the nerve rings at the points where the branches from the central system come into the ring (Fig. 3 in the text). Every point of connection of the ring with the central system is also a point of junction with two long bipolar cells. I regret exceedingly that the histological character of this junction is wholly obscured by the swelling of the tissues by the formic acid, and I hope to study this detail by the use of Golgi's method of silver impregnation or methylen

blue. The bodies of the cells lie about midway between the nerve rings (Pl. V, long *bpc.*), and present the same general appearance in size and nucleus as the bipolar and "Leydig's cells."

The physiological rôle played by this highly specialized peripheral system is, doubtless, of the utmost importance, and anything like a discussion of its functions can be made only after the details of its constitution have been more fully worked out, and something is known of the comparative anatomy of the structure in other worms. It plainly offers a method for short reflexes, such, for instance, as those controlling the rhythmic undulating motions of the leech when at rest, and supposed to be respiratory; or for the successive stimulation of the muscles in voluntary motions. The presence of this system may throw some new light on the phenomena that go under the name of "skin tension."

Leaving its physiological functions for another investigation, its presence may be brought to bear testimony on the question of the derivation of the five-ring form of metamere. In his *Metamerism of Clepsine*, Whitman says (p. 392): "In my description of *Clepsine plana* (1891) the following note may be found (p. 414): 'I am reminded of an error into which I fell in my paper on Japanese leeches. The error was the assumption that all somites having less than five rings were abbreviated. The assumption should have been, as I now feel convinced, that all somites with less than three rings are abbreviated, and all with more than three have been increased by the division of one or two of the three primary rings. I have collected considerable evidence, which cannot be given here, to show that in the evolution of Hirudo it was the *2d and 3d rings that underwent division*, while the 1st remained undivided.'" On page 393 he continues, under the head of "Multiplication of annuli": "It is a fact of some importance, in estimating the morphological value of the metamere, that the multiplication of annuli seems to follow the same general law as the multiplication of metameres in the embryo; that is to say, the *posterior end* of the metamere, like that of the embryonic trunk, is the region of most rapid growth and elon-

gation, and the new rings are added by the division of the ultimate (3d) ring alone, or by the division of both the ultimate and the penultimate, somewhat as new metameres are added by the division of the part lying behind the last one formed. There is not then a uniform growth throughout the trunk, but a curve for each metamere."

When I first found the intermuscular nerve ring I made a very careful search in the 3d and 4th annuli for every trace of nerves, and found that they were very weak in those structures, and when I came to plot down the nerve ring as it occurs in the successive metameres, it became evident that the 5th and 2d annuli of each somite (see Pl. V) were in strong and equal connection with the 1st annulus which carries the ganglion.

The absence of anything like a proportional division of nerves between the several annuli shows, I believe, that the annuli weak in nervous elements are the younger and secondary annuli, formed by the division of the 2d and 3d rings as follows: the posterior half of Clepsine 2 becomes 3 in Nephelis, and the anterior half of Clepsine 3 becomes 4 in Nephelis. This mode of formation of the five-ring type of somite does not involve any shifting of the nephridiopore, as would happen if the posterior half of primitive 3 became 5 in Nephelis.

This explanation assumes, of course, that the intermuscular nerve ring is a constant feature in the leeches, and I feel confident in predicting its discovery not only in the leeches, but either that or its homologue in other annelids as soon as they are studied with good nerve methods. There are many evidences in the structure of the ring that it is an old and very stable structure. The constancy through successive metameres of such features as a long pedicelled cell always in the same position on the ring ; the group of sensory fibers separated from motor fibers near the inner ventral group of bipolar cells ; the strong innervation from the central system, and the definite longitudinal connectives, all point strongly to the nerve ring, as we find it in Nephelis, as being very highly specialized and the resultant of two originally distinct systems.

In Lumbricus, for instance, both the afferent and efferent fibers of the central system, though somewhat more diffuse than in Nephelis, run in well-defined bundles, and Miss Langdon (17) has found numerous bipolar cells along these nerves. These two elements, fibers and cells, necessary to form the intermuscular nerve ring of Nephelis are present in Lumbricus, and when one takes into consideration the vast differences between the life habits of the sluggish, mainly herbivorous, earthworm and the active, free-swimming, carnivorous Nephelis, it does not seem difficult to believe that specialization, so far advanced among the leeches in other particulars, may so combine these factors as to produce the result found in Nephelis. This suggestion by no means excludes any other explanation; it is the one nearest at hand in the light of our present knowledge. Recent investigations, with methylen blue especially, show that the peripheral bipolar ganglion cells connected with the central system play an important part in the neural system of some of the flat worms, and the whole matter of peripheral nerve systems in this group, as well as that of the annelids, is now in such a promising condition of investigation that much light will, doubtless, soon be thrown upon it.

## The "Large" Nerve Cells.

Investigators of annelid and arthropod nervous systems have been familiar for a long time with certain nerve cells so large in comparison with the ordinary motor cells of a ganglion that they have often designated them by such words as "giant," "colossal," and the like, and have described their location, character, etc., without, so far as I know, bringing them into any relation with each other. Such a relation exists in Nephelis, though what its full significance may be I do not yet know. I find the large cells in a body somite arranged as follows: in the ventral chain two "median" or "giant" cells *within* the ganglion; in each connective *between* the ganglia lies a "colossal" axial cell, which sends processes before and behind into

[17] Fanny E. Langdon: "The Sense Organs of Lumbricus agricola Hoffm." *Journ. of Morph.*, vol. xi, 1895.

the ganglion; therefore, *near* the "median" cells. In each intermuscular nerve ring are about twenty-two "large bipolar" cells, some of which send processes *into* the ganglion in the neighborhood of the "median" cells, while the eight "connective" bipolar cells joining the two rings terminate in some manner in close proximity to the fibers of the nerve ring at their junction with the ring. This leaves but one "large" cell yet to be accounted for, the "Leydig's cell" near the ganglion. This is a bipolar cell, and the processes may be readily distinguished as they pass outward on each lateral nerve *toward* the periphery. These processes, however, soon fuse with the nerve trunks, and I have not been able to follow them to any considerable distance. It is quite significant that these same trunks, or branches from them, send fibers into the intermuscular nerve ring, and furnish a path by which the processes of this cell may reach to the other large cells. If it does not come into proximity with the others, it forms an exception to all the other "large" cells in the somite.

Another fact to be noted of all these cells, numbering nearly fifty in each somite, is that the nuclei are practically the same in size and character, and the volume of the different cells is, so far as sections show, practically the same, whatever the shape may be. Such a definite arrangement cannot be without a purpose, the significance of which may in some measure be revealed by their development, and this I hope to determine soon.

In brief, if by some means we could remove all other cells and tissues in a somite excepting the "large" nerve cells and leave them in their normal relations, we should find them all joined together, forming a closed system capable, on the one hand, of receiving impressions and stimulating muscles independently, and, on the other hand, so related to the central nervous system through the cells in the ganglia and connectives as to make it completely adjunct to it.

### THE SYMPATHETIC SYSTEM.

Leydig (18) and others have found evidences of a sympathetic system arising from the collar in certain leeches and other

[18] F. Leydig: Tafeln zur vergleichenden Anatomie. Tübingen. 1864.

annelids. In Nephelis, I have found it to be much more extensive than has been figured in any annelid that has come to my notice.

It arises in Nephelis very similar to the method figured by Leydig (*l. c.*) for *Haemopis vorax* Brandt (Pl. IV, Fig. 5). In this latter leech Leydig shows the sympathetic system lying on the walls of the "crop," but not connected with the part arising from the collar. In Nephelis (Pl. VII, Fig. 17) the junction between the two systems is on the median side of the collar near the nerve root I–II. A fibrous projection from the anterior side of the collar on each side gives rise to three branches which run over the wall of the oesophagus; the dorsal and ventral roots pass off in a ⊣ fashion, while the lateral root comes off from the median side. Six capsules, three on either side, contain nerve cells whose processes run into these branches. One pair (Pl. VI, Figs. 11 and 13, symp.), the larger of these capsules, is on the collar, the other two on the posterior side of the dorsal branch. The ventral branches retain their individuality for some distance as they approach the mid-ventral line, but they soon become lost in a system of closed meshes. The lateral branches continue as such, plainly taking part in forming the meshes, but preserving their identity throughout (Pl. VII, Fig. 17; Pl. VIII, Fig. 19).

The dorsal branch of each side rises parallel to the collar, just in front of it. Professor Patten has called my attention to the fact that this structure in this position is comparable to a similar structure in Limulus. A narrower band connects them in the mid-dorsal region so that they form together a half circle. Two branches pass backward near the median plane into two large ganglionic masses lying just under the collar (Pl. VIII, Fig. 20). All these branches give off bundles of fibers that run forward to the buccal cavity, and these bundles differ in two ways from the plexuses behind the collar. They contain but few, if any, cell bodies, being processes of the cells that lie clustered together in ganglionic masses between the fibers and the meshwork of the plexuses on the oesophageal walls. Fig. 20 shows this as it occurs on the dorsal side. It is a dorsal view drawn from a Haller preparation and, hence, shows no

cells. The same characteristic difference between the fiber bundles which run forward and the plexuses is found at each of the other branches, and similar but smaller ganglionic masses are present. Back of the collar, the muscular wall of the alimentary canal is covered with a complicated meshwork, as shown in Pl. VII, Fig. 17, and shown in greater detail in Pl. VIII, Fig. 20. The cells are multipolar and send processes in various directions, forming meshes. The processes ramify the wall and innervate the muscle cells. The preparations made with formic acid are not satisfactory for histological detail, and Fig. 20 is introduced to show the distribution, not the structure.

This system continues over the whole alimentary tract in substantially the same manner as shown on the oesophagus, and though from theoretical considerations I expected and sought diligently for metameric connections from the central system, I am confident that none exist.

In a few very favorable sections I have seen what I believe are traces of the sympathetic system in the post-anal region, extending in the axial line of the acetabulum and the sucker. The musculature in this region is so complicated that I cannot determine this point to my complete satisfaction. Nerve cells and fibers are certainly there and show plainly. There is no theoretical objection to their being a part of the sympathetic, for if in the ancestral form the anus was terminal and the sympathetic system was present to the anus, then in the leech the formation of the sucker undoubtedly made demands upon the muscles of the alimentary tract that may have continued after the anus moved forward and the sucker became imperforate. Again, while feeding, the leeches always hold themselves fast by the sucker, and the stronger stimuli to the muscles excited by food in the alimentary tract, during a meal, may by this same system be communicated to part of the muscles of the sucker and may help to make the adhesion more effective.

## Summary.

1. Nephelis differs from nearly all other leeches in the external topography of the somite. The prominent sense organs present in most genera are not easily visible in Nephelis, excepting a few somites in the anal region.

2. Color and color markings do not afford criteria for the determination of specific characters.

3. The body somites consist of five annuli as determined by the nephridiopores. The somites of the terminal regions, containing less than five annuli were determined by the innervation.

4. But one species of Nephelis came under my observation though the collections were made over a wide area of country.

5. The food supply is the controlling factor in the choice of location in a pond or brook.

6. The head region contains six somites; the body region, eighteen; the anal, ten, making thirty-four in all.

7. The anterior nerve of a body neuromere arises from two roots which fuse quickly; the anterior nerve is, therefore, the morphological equivalent of the first and second nerves of Clepsine, while the posterior nerve in Nephelis is the equivalent of the posterior nerve in Clepsine.

8. "Leydig's cell" is present in every neuromere from one to thirty-four.

9. The innervation of a body metamere in Nephelis is morphologically identical with that of Clepsine.

10. The three anterior neuromeres of the anal ganglia show evidences that reduction in Nephelis has progressed by so much more than Clepsine, whose anal ganglia are represented by the succeeding seven neuromeres. In the head region the number of neuromeres is the same in both.

11. The distribution of the nerves in the somites of the terminal regions is precisely referable to that of a body somite.

12. In both terminal regions each neuromere contains every element found in a body neuromere.

13. A peripheral system of nerves composed of large bipolar cells, which I have called intermuscular nerve rings, is in intimate connection with the central system.

14. Some of these cells supply the fibers which Retzius and Biedermann describe as ending in the ganglion.

15. These rings are connected longitudinally by other bipolar cells which thus form direct axial paths for nervous impulses in addition to those furnished by the central system.

16. The relation of these rings to the distribution of nerves in a body metamere affords striking proof in favor of Whitman's theory of the formation of a metamere with five annuli from one of three annuli, that is, the posterior half of annulus 2 in Clepsine becomes annulus 3 in Nephelis; the anterior half of annulus 3 in Clepsine becomes annulus 4 in Nephelis, while the posterior half becomes annulus 5.

17. There is some evidence that this peripheral system of nerves is an old and very stable structure, and will be found in the other leeches. There is already some evidence that it or its forerunner is present in other annelids.

18. The "giant" nerve cells in the two systems, central and peripheral, are in close relation to each other, and are strikingly alike in cytological characters.

19. The sympathetic nervous system is well developed, and is connected with the central nervous system at the "collar."

20. The branches at this connection form a nerve circle in front of the "collar," such as is found in the arthropoda.

21. The nerve cells in the sympathetic system are multipolar, the processes forming meshes over the wall of the alimentary tract.

22. There is some evidence that the sympathetic system persists in the post-anal region, extending in the axial line to the muscles of the concave side of the sucker.

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## DESCRIPTION OF PLATE IV.

Reproduced through the kindness of Professor Whitman from the "Festschrift zum siebenzigsten Geburtstage Rudolph Leuckart's," Leipzig, 1892, being Pl. XXXIX of that volume and appended to Professor Whitman's article, "The Metamerism of Clepsine."

Representing the first eight segments as reconstructed from sections and surface views. × 50. The segments and nerves are numbered with Roman characters, the annuli with Arabic numerals. The metameric and smaller accessory sensillae of the dorsal side are represented in black, on the ventral side by circles.

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## REFERENCE LETTERS.

*Alphabetically arranged.*

*ant.n.*, anterior nerve. — *b.*, buccal annulus. — *d.b.*, dorsal branch of post. nerve. — *d.b. IV a. V*, dorsal branch of nerves IV a. V. — $E^1$, rudimentary eyes. — $E^2$, principal pair of eyes. — *f. 1* and *2*, nephridial funnels. — *g.d.*, bundles of gland ducts. — *i.l.*, inner lateral sensillae. — *lab.gl.*, labial glands. — *l.s.*, longitudinal septa. — *m.*, median sensillae. — *mg.*, marginal sensillae. — *mg.s.*, marginal sinus. — *m.s.*, median sinus. — *mid.n.*, middle nerve. — *n.c.*, nerve cord. — *neph. 1–2*, 1st and 2d nephridia. — *neph.p.*, nephridial pores. — *o.l.*, outer lateral sensillae. — *p.b.*, post-buccal annuli. — *ph.gl.*, pharyngeal glands. — *post.n.*, posterior nerve. — *s. 1–4*, septa. — *s.n.*, septal nerve branch. — *tr.s.*, transverse sinus. — *V 1–2*, funnel vesicles. — *v.b.*, ventral branch of posterior nerve.

## DESCRIPTION OF PLATE V.

Dorsal view of the central nervous system of Nephelis in the first seven and part of the eighth metameres as reconstructed from surface views and sections. × 30 (circa). The metameres are indicated on the left of the drawing by Roman numerals, the annuli are numbered by Arabic figures on the right. The nerves are also numbered with Roman numerals. Dorsal sensillae appear as black circles; ventral sensillae as light circles, excepting in the first five annuli, where they are drawn as they appear in a surface view of a specimen killed and examined in Haller's fluid. The details of distribution are shown so plainly as to do away with the need of explanatory references. On the left side, omitted from the right for clearness, are seen the long bipolar cells (long *b.p.c.*) that connect the intermuscular nerve rings. They are found at the junctions (Junctions) of the nerve branches with the rings, and the cut end of these branches are represented on that side. Faivre's nerve is indicated at *F.N.* The first pair of nephridiopores (*1st nph.p.*) lie between the 16th and 17th annuli.

## DESCRIPTION OF PLATE VI.

FIG. 3 is a diagram of *Nephelis lateralis* showing the boundaries of the somites in Roman numerals, the annuli in Arabic figures, the nephridiopores (*nph.p.*), the boundaries of the clitellum (*cl.*), and the sexual openings (male ♂, female ♀). The "brain" and the first two body neuromeres are sketched in to show their relative positions.

FIG. 4 is a dorsal view of *N. lateralis* from Wolf Lake near Chicago, Ill., showing the relative size of the annuli, and the number of sensillae as seen in a specimen freshly killed in weak chromic acid. The eyes are represented by crescentic black areas, as seen in a specimen killed in Haller's fluid. They represent the pigmented part only, the large, clear visual cells extending in a cone-shaped cluster from the concave side. The first pair look forward and outward, the second and third pairs backward and outward. Camera drawing: Zeiss, comp. oc. 1, obj. AA.

FIG. 5. Ventral view of the same showing the oral sucker as it appears in a freshly killed and well-extended specimen. Annulus 3 is the first complete annulus.

FIG. 6. Lateral views of the same showing the incomplete separation of annuli 1 and 2 and the doubling often seen in annuli 4 and 6.

FIG. 7. Anal region, dorsal view. Annuli 97, 102, 104, and 106 show well-marked sensillae in specimens freshly killed in weak chromic acid, though the number is not constant. Annulus 104 is double, the posterior half bearing the sensillae. The semicircular area on top of the sucker, the "acetabulum," is finely wrinkled, all traces of annulation being lost.

FIG. 8. Lateral views of an unusual specimen showing the partial fusion of the narrow annulus 5 with the broad annulus 4. Wolf Lake.

FIG. 9. Dorsal view of a normal body neuromere, showing the elliptical fibrous part in the middle, the two perforations in it near the median line, the slight groove in the median line passing between them. Faivre's nerve is shown as a narrow line between the two connectives. Two pairs of lateral capsules containing nerve cells lie on either side of the fibrous portion, one lying anterior to each of the lateral nerves. Two ventral capsules are shown under the fibrous portion. The dorsal and ventral roots of the anterior lateral nerve are shown together with the "Leydig's cell" lying between the two lateral nerves. (Nitric acid maceration; slightly stained with borax carmine.) Camera: Zeiss, obj. A, oc. 3.

FIG. 10. Dorsal view of the "brain," the sub-oesophageal ganglia, and first body neuromere, VII. (Nitric acid maceration.) Camera: Zeiss, obj. AA, oc. 2.

FIG. 11. Tracing of same. The capsules are numbered to correspond with the neuromeres to which they belong. The capsule designated "Symph" contributes its fibers to the sympathetic system only. It is not metameric.

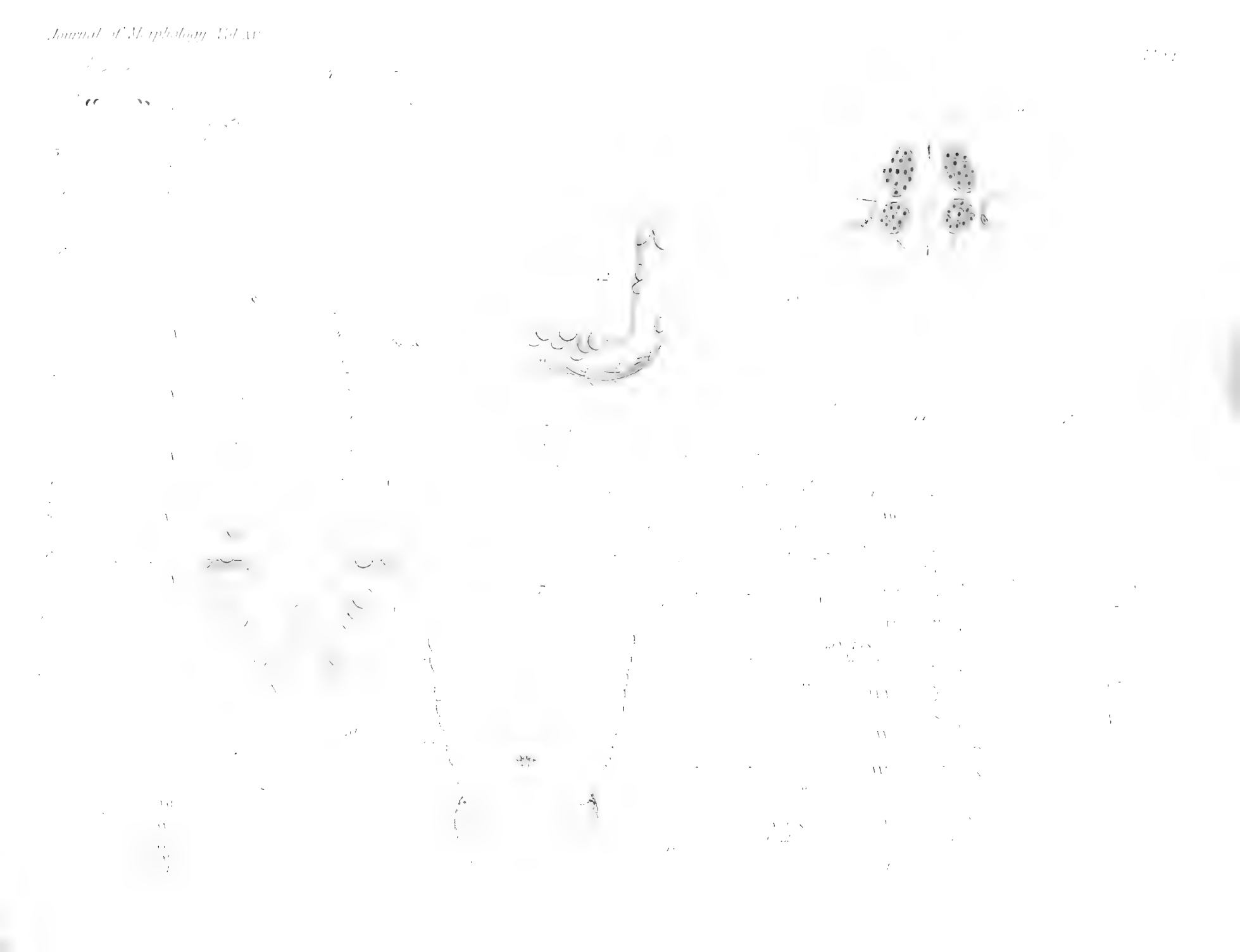
FIG. 12. Side view of "brain" of same specimen, with same magnification.

FIG. 13. Tracing of same; capsules numbered as before. This view shows the relations of the lateral capsules to the roots of their respective nerves in the "brain," and the same relations as they exist in the body neuromere, VII. The separation of the lateral capsules, 3. 3., of neuromere III is also shown. The position of the junction between the "brain" and the sympathetic is indicated on

the median side of the collar at the level of the root of the nerves I, II. One large and two small capsules are connected with the sympathetic at each junction, which sends a dorsal and a ventral nerve bundle, shown here, and a lateral bundle not shown here, to the muscular wall of the alimentary canal. See Pl. VI, Fig. 17.

FIG. 14. "Anal ganglia," dorsal view.

FIG. 15. Ventral view of same. Metameres in Roman numerals. Capsules in Arabic figures. In Fig. 14 the crowding of the lateral capsules to the dorsal side begins with neuromere XXVIII, while the ventral capsules, Fig. 15, of the same neuromere are also crowded out of the normal. The seven neuromeres, XXVIII to XXXIV, constitute the entire "anal ganglia" of Clepsine. XXV is nearly normal, lateral nerves not fused; XXVI is slightly compressed, lateral nerves fused, but the two roots are plainly visible; XXVII is still more compressed. These three neuromeres indicate the extent to which abbreviation has proceeded farther in Nephelis than in Clepsine in this region.



## DESCRIPTION OF PLATE VII.

FIG. 16. Lateral view of the nervous system of the head region, reconstructed from sections, showing the distribution and general paths of the principal nerve trunks, the intermuscular nerve rings in annuli 5, 8, and 11, together with their junctions with the central system, and other details. The principal eyes lie in annulus 2, the small ventral branch of nerve II passing through it axially to the labial sense organs. The second and third eyes look to the rear. On nerves I, II, III, and IV the " Leydig's cells " of those neuromeres lie outside of the nerve trunks which are the fused anterior and posterior lateral nerves of their respective neuromeres. In nerves V and VI the same cell is seen near the angle of separation of the fused parts.

FIG. 17. A reconstruction showing the general arrangement of the sympathetic system. Combined from sections and specimens killed in Haller's fluid. The body outline is drawn from a median section, the same as used in Fig. 16, and the parts of the central nervous system are traced in outline from Fig. 16. The right half of the oesophagus is shown covered with the closed meshes of the plexuses formed by the fibers from the multipolar cells. The cells are inadequately shown; see Fig. 20, Pl. VIII. Standing out from the median side of each half collar, median to the fused roots of nerves I, II, is a fibrous projection which gives rise to three trunks: (1) one going ventral and median, meeting the similar trunk of the other side in the mid-ventral line; they form anastamoses and gradually lose their outlines in the general plexus; (2) a lateral trunk that persists to the rear as shown; (3) a dorsal branch which runs parallel with the collar to the median dorsal line; the details of this arrangement are shown in Fig. 20. The dorsal trunk of each side carries two small capsules containing nerve cells, while a third capsule, whose fibers go into the sympathetic system, is located on the collar, not far from the origin of the connection between the central system and the sympathetic. Anterior to the collar the sympathetic system runs to the buccal cavity in fibrous bundles, apparently free from cell bodies.

## DESCRIPTION OF PLATE VIII.

FIG. 18. A projection of the nerves of the fifth and following first annuli showing especially the intermuscular nerve ring and its relations to the central nervous system. On the ring are ten (five in the half section) groups of bipolar cells designated as below. The trunks from the central system join the ring at the points marked 1 to 4 on the margin, which points also mark the points of junctions of the long bipolar cells with the ring. The numerals in the figure designate the bundles of longitudinal muscles, here seen in section, and drawn only in three of the ventral bundles. In these are shown fibrillae which leave the ring to innervate the muscle cells. The layers of circular and oblique muscles between the epidermis and the long muscles are omitted for clearness, in order to show the fibers from the sensillae to the ring.

---

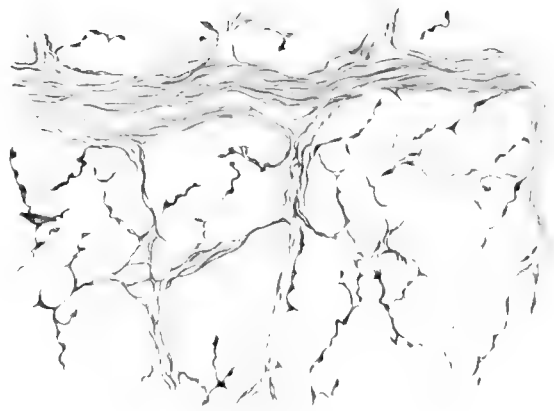
### ABBREVIATIONS.

| | |
|---|---|
| *al.* | intestine. |
| *br. to 1st ann. et seq.* | branches of nerves to the respective annuli. |
| *epi.* | epidermis. |
| *gn.* | ganglion. |
| *l.v.* | lateral blood vessel. |
| *med.bdl.l.m.* | median bundle of long muscles. |
| *m.circ. and ob.* | layer of circular and oblique muscles. |
| *sens. I, II, III.* | three types of sensillae. |
| *in.v.b.p.c.* | inner ventral group of bipolar cells. |
| *o.v.b.p.c.* | outer " " " " |
| *in.d.b.p.c.* | inner dorsal " " " |
| *o.d.b.p.c.* | outer " " " " |
| *lat.d.b.p.c.* | lateral " " " " |
| 1–4, marginal. | junctions of ring with central system and the long connective bipolar cells. See Pl. II, Junctions. |
| 1–6, within. | ends of the bundles of long muscles. |

Outlines taken from camera drawings.

FIG. 19. Camera drawings of a section showing details of the sympathetic system. The faint circles are the cut ends of muscle cells. In many of them may be seen the nerve end plates. The wide bundle of fibers is part of the lateral bundle. See Pl. VII, Fig. 17. Reichert, $\frac{1}{12}$ immersion, oc. 3.

FIG. 20. Dorsal view of part of the collar and sympathetic system. From a flattened head killed in Perenyi's fluid and viewed as a transparent object in Haller's fluid. It shows the continuity of the dorsal branches of the sympathetic, the ganglionic masses under the collar (Pl. VII, Fig. 17), and the different character of the fibrous bundles running to the buccal cavity and the plexuses posterior to the collar. Zeiss, c. oc. 2, camera drawing, reduced nearly one-half.



# THE GROWTH OF THE OVUM, FORMATION OF THE POLAR BODIES, AND THE FERTILIZATION IN POLYCHOERUS CAUDATUS.

EDWARD G. GARDINER.

WHILE studying the segmentation of the ova of *Polychoerus caudatus*, large numbers of these little animals were kept alive in small aquaria under constant observation, and their habits were carefully studied. When a number of them are placed together in a small dish, after a short time most of them settle on the bottom of the dish and remain quiet. There are generally, however, a noticeable few who keep on the move, endeavoring to find a lodgment on the back of some other individual. Sometimes one of the latter may remain motionless and allow a pursuer to creep onto its back, but not infrequently it moves off a short distance and then comes to rest again, in which case the disturber follows and again endeavors to gain a resting-place on its back. I have seen one of these worms thus pursue others for upward of half an hour, and each time just as it gained a lodgment on the back of the one sought, the latter would move off. Finally, as if to get rid of further annoyance, the pursued one came to the surface of the water, where it swam with its ventral side up, thus preventing its pursuer from accomplishing its purpose. After these observations had been repeated many times, it occurred to me that the pursuer was endeavoring to fertilize the pursued by hypodermic impregnation. To confirm this I have taken one of these restless individuals and put it, with several others which were at rest, in a shallow watch glass, where they could be observed more closely. I found that after the restless one gained a lodgment on another both were quiet for a short time and then separated.

More frequently the under one moved first, and then in such a manner as to suggest that it had been suddenly disturbed by its burden. Several times I have seen the under one suddenly

give convulsive struggles as if to rid itself of its annoyer and swim rapidly away. Such individuals, when killed immediately, showed in sections spermatozoa adhering to the back and penetrating the tissues. In several of these specimens the surface of the back where the spermatozoa were *en masse* was slightly abraded. Whether or not this abrasion was caused by the action of another individual or was due to accident is uncertain. It seems probable that the numerous chitinous "mouth pieces," which are one of the characters on which Mark (1) has founded the genus *Polychoerus*, may be used by the animal to pierce the skin of other individuals so that spermatozoa deposited thereon may penetrate. The penis of this form is unarmed, and is situated a short distance behind these "mouth pieces," to which no definite function has hitherto been attributed. That this is the normal method by which fertilization is effected in this species I have no doubt whatever, and believe it is but another case of hypodermic impregnation to be added to the long list cited by Whitman (2) in his paper on this subject. In no case, except when intertwined and dying in stale water, have I seen two individuals bring their ventral sides in contact as would be necessary in copulation. In this group fertilization always takes place before the ova are laid, and Dr. Sophie Pereyaslawzewa (3) states that in all cases the polar bodies are formed while the ova are still in the parent. That this is normally the rule in this species will be shown later.

In specimens examined under the slight pressure of a cover slip it is easy to determine whether or not the eggs have been laid, for when present they can be distinctly seen each with its large round germinal vesicle. Very frequently, however, instead of the germinal vesicle, a clear, translucent, dumb-bell-shaped structure, which occupies the greater portion of the ovum, may be seen. This is the amphiaster of the first segmentation spindle, which in this species is usually formed before oviposition. This is, however, not an invariable rule, for I have found, in normal egg capsules, ova with round, intact, germinal vesicles. In such cases, when the polar bodies are formed they are always extruded from the egg, while when

they are formed before the egg is laid they are always retained within it. Further, it was noted that, when first captured, animals are more apt to lay than when long in captivity, even under the most favorable conditions of fresh sea water, etc., that I could devise. When, however, they are kept long under rather unfavorable conditions, such as slightly stale or too warm water, the dumb-bell-shaped structure disappears by the drawing together of its extremities, and the nucleus *appears* to return to its resting-stage.

Sections show, however, that the true resting-stage is never attained. The centrospheres still exist fairly distinctly, each containing in its center a faint centrosome. The cytoplasmic network, which, when the spindle is fully formed, is so startlingly conspicuous, has disappeared, and the achromatic spindle fibers have become very indistinct and shortened. The chromosomes have lost their cheveron shape, and in some cases seem to have melted so as to form round bodies, and the outline of the whole structure is but slightly oval, and occupies very much less space than does the fully formed amphiaster. In this stage, when examined in the living specimen, this retrograde amphiaster may readily be mistaken for an intact nucleus. Sections show that it is but poorly defined in outline, and apparently the greater portion of the material which formed the amphiaster has changed its chemistry, so that it no longer differentiates by stains as formerly. To what extent this degeneration can take place, without the power of recovery being destroyed, it is impossible to say, but the ova of worms which have been kept for a long time under such abnormal conditions sometimes fail to develop.

This very unusual action of a spindle once fully formed suggested immediately the "eigenthümliche Art der Kernmetamorphose," noted first by Selenka (4) in the ova of Thysanozoon, and afterwards in other forms by Lang (5) and Wheeler (6). Selenka describes in the uterine ova of *Thysanozoon diesingii* a spindle which must strongly resemble in its action that just described in *Polychoerus caudatus*. To quote him directly, he says: "Nachdem das Ei seine definitive Grösse erreicht hat, beginnt das Keimbläschen sich in typischer

Weise zur Theilung anzuschicken: die chromatischen Kernfäden (ich gebrauche hier und in der Folge die Bezeichnungen welche Flemming eingeführt hat) ordnen sich zur Knäuelform, die achromatische Fadenspindel mit ihren Polarkörpern, die zwei Radiensysteme der Eikörperstrahlung treten auf u. s. w. Sobald aber die Fadenschleifen des Kernes die 'Sternform' oder die Form der sog. Aequatorialplatte erlangt haben, sistirt die begonnene Kerntheilung, und indem die vorher weit auseinander gerückten Polarkörper sich langsam wieder nähern, verschmelzen auch die Fadenschleifen wieder zur 'Knäuelform,' die Dotterstrahlung verschwindet nahezu gänzlich und der Kern kehrt zur Ruheform zurück. Der letztere unterscheidet sich von dem früheren Keimbläschen durch die centrale Lage im Ei und den Mangel eines grossen Keimfleckes. Der ganze Prozess kann also mit einer auf halbem Wege stehen gebliebenen und wieder rückschreitenden indirecten Kern- und Zelltheilung verglichen werden. Ein Resultat dieses Vorganges ist leicht zu erkennen: nämlich die Umgruppirung der Dotterkörnchen. Während diese Dotterkörnchen anfänglich gleichmässig im Dotter zerstreut lagen, werden sie durch die erwähnten Vorgänge um die Centren der beiden Astera geschaart und durch Annäherung der letzteren endlich in die Mitte des Eies geschafft."

In discussing this phenomenon, Lang says (pp. 295, 296): "Die im Uterus enthaltenen Eier aller von mir untersuchten Cotyleen und viele Acotyleen zeigen eigenthümliche Veränderungen ihres Kernes, die vollständig mit denen übereinstimmen, welche der Kern erleidet wenn sich die Zelle zur Theilung anschickt. Ich kenne diese Veränderungen schon seit vielen Jahren, vermochte aber nie für dieselben eine befriedigende Erklärung zu finden." Further, while Lang has no serviceable hypothesis for the *raison d'être* of this phenomenon, he utterly declines to accept that offered by Selenka, *viz.*, that it is a normal action of the nucleus to bring about a redistribution of the "Dottermaterial" in the ovum. Still, Lang regards it as a normal process of the nucleus, and does not connect it with the conditions under which the animals bearing the ova may have been brought just before being killed, as I think should be done.

While seeking to compare the above described retrograde growth of the amphiaster in the ova of an Acoela with the "disappearing spindle" in the uterine ova of a Polyclad, the following facts should be borne in mind. In both of these groups the spermatozoa to be used in fertilizing the ova are contained within the egg-bearing animal, introduced, probably, in most if not in all cases, by hypodermic impregnation. (See Lang, p. 636.) In the Polyclads the normal process is that, when the eggs are laid, one or two spermatozoa pass with each ovum into the egg capsule, where fertilization takes place later; hence, if for any reason oviposition is delayed or prevented, it would seem perfectly possible that fertilization might be effected *in utero*. That such is the case under some circumstances the following experiments show conclusively.

In the summer of 1895 and again in 1896 I obtained quite a number of *Leptoplana variabilis* (Verrill), which laid quite freely in captivity. In each egg capsule examined, from one to three spermatozoa were found. In less than three hours after oviposition the polar bodies were extruded, and in from one to three hours after this the line of the first cleavage plane was evident, and the two-celled stage was soon after reached.

These facts being established, I brought six specimens which appeared to contain ripe ova into a dish of warm, somewhat stale sea water, in which were a number of *Polychoerus caudatus* on which I was experimenting. Within an hour two laid ova in which the development proceeded as above described. At the end of eight hours the ova in the other four had, as far as I could observe, undergone no change. The next morning (twenty hours) the worms were almost dead; nor did they appear to revive on being placed in fresh sea water. They were then killed and sectioned. In two ova amphiasters were fully formed, which from their small size appeared to be destined for polar body formation rather than for the first segmentation cleavage. Into one of these ova the sperm had penetrated. In another ovum a polar body had been divided off, but not extruded, and a sperm had entered the ovum; while in another, two polar bodies were lying close to the egg membrane, and

the first cleavage spindle was forming. These experiments were repeated several times with about the same results.

The sections in no case gave very satisfactory preparations, for frequently the tissues and the ova were abraded and injured by the severity of the experiment. It was, however, clearly demonstrated that if the animal bearing ripe ova were placed under such conditions that it either could not, or would not, lay its eggs, the development of the ova continued much as if normal oviposition had taken place.

In several ova a larger spindle strongly resembling the disappearing spindle as shown by Lang (Tafel 20, Fig. 4) was found. I would suggest, therefore, that individuals of the Polyclads, in which such structures are found, have before death been placed under some abnormal conditions ; that the ovum has been fertilized and the polar bodies formed ; that the first segmentation spindle has been formed ; and that the environment was such that oviposition could not take place ; consequently, that a retrograde development of this spindle has taken place exactly as in *Polychoerus*. If this is so in one Polyclad, it may certainly be so in others, and it seems much more logical to assume that under fitting conditions the first segmentation amphiaster may be formed in the uterine ovum and then undergo retrograde development, as I have demonstrated to occur in *P. caudatus*, than that an amphiaster should be formed with the express purpose of disappearing again, as the observation above quoted would indicate. The figures in von Graff's (9) great work show in animals of several species ripe ova with large amphiasters already formed within them. Von Graff, however, does not discuss this matter in the text. It is interesting to note the parallelism in the extrusion or non-extrusion of the polar bodies in these two forms. In both, the polar bodies are extruded only when the ovum has been laid before these bodies are formed. If, for any reason, the polar bodies are formed before the eggs are laid they are not extruded. It may be that the sea water comes in more intimate contact with the egg in the capsule than when within the parent, and in some way stimulates the ovum to extrude the bodies.

### *Growth of the Ovum.*

Several methods of killing and hardening were used, but by far the best results were obtained after using Hermann's fluid. No other reagent seemed to preserve the nuclear structure so satisfactorily. Pretty good results were also obtained with Flemming's fluid, and also with weak formaline, but corrosive sublimate solutions, either with or without the addition of acetic acid, gave but poor results. As related in a former paper (10) on the early development of this form, no satisfactory method of killing the ova after they were laid in their capsules was found. This is probably due to the impenetrability of the capsule.

In his monograph on the Rhabdocoela, von Graff (8) states that in the Acoela no vitellarium is present; and in this Dr. Sophie Pereyaslawzewa (3) agrees. In a later work (9) he describes with some detail the rapid growth of the ovum from the small germ cell as being due to one germ cell absorbing the substance of its immediate neighbors, which are thus continually reduced in size, while the only germ cells destined to reach maturity grow at their expense. Thus, he says, a struggle for existence and survival of the fittest occurs among germ cells just as among individuals. That this is the method of growth of the ova in many Acoela I have satisfied myself by sectioning specimens of different species; but in *P. caudatus* it is quite different, for, when very small, the ova pass from the ovary into an enlarged and differentiated portion of the oviduct, which is charged with food material, which the ova there absorb. Professor Mark (1) speaks of this as a "differentiated portion of the ovary, where the cells destined to reach maturity undergo . . . rapid increase in size." I think this should be described rather as a differentiated portion of the oviduct; for, as Pl. IX, Fig. 1 (*vt.*), shows, it lies between the ovary and the female genital pore. This Fig. 1 is a diagram showing the condition of the female reproductive organs at different seasons. The left half shows the ovary (*ov.*) and enlarged oviduct filled with vitelline or food material (*vt.*), in the spring or early summer, before the near approach

of the breeding season. The right half of the diagram shows the same parts when the ova are rapidly approaching maturity. The ova (*o.*) which are destined for fertilization are contained within the vitellarian portion of the oviduct (*vt.*). These are all about the same size and appear equally sure of surviving, for all the ova in this gland are laid at the same time. Extending forward and at the same time upward from this vitellarium is the ovary.

With but slight magnification three distinct stages in the breeding season may be distinguished among individuals. Those approaching the breeding season have enlarged vitellaria which do not contain ova; later the vitellaria are crowded with enlarging ova, and again later, after the ova are deposited, the vitellaria are completely collapsed. I have kept animals for several weeks after they have laid to determine whether or not they laid a second time during the same season, but have found no evidence that they do so. The vitellarium did not recover from its collapsed state.

Double staining with lithio-carmine, followed by Lyons blue, after a method described by Miss Katharine Foot (11), shows that the substance of the vitellarium is very different from that of the ovarian ovum. In the latter, both nuclei and cytoplasm take the red stain with great avidity, but do not stain at all with the Lyons blue. On the other hand, the greater part of the vitellarium is indifferent to the lithio-carmine, but does stain with the blue. Sections through the vitellarium (Pl. IX, Fig. 3) show it to be composed of large cells, the nuclei of which are closely pressed against the cell walls. The nuclei, cell walls, and a few fine protoplasmic filaments within the cell stain red, while the cell contents stain a strong blue. Fig. 2 shows a section through a portion of the vitellarium with three ova imbedded in it. The ova have evidently just passed in from the ovary, for they do not take the blue stain. Fig. 4 shows an ovum which is about mature, having absorbed the dotter material from the surrounding gland cells to such an extent that the red staining cytoplasm, of which it was composed when it first entered the vitellarium, is completely obscured. Around it are the collapsed and empty gland cells.

In the ova of *Allolobophora foetida* Miss Foot found that the blue stain attacked the archoplasm and the spindle fibers, while in the ova of *P. caudatus* its greatest affinity seems to be to the lifeless dotter material, both while in the vitellarium and later while in the ova. It is curious that these stains should act so differently on the ova of different animals. It is unfortunate that this lithio-carmine and Lyons blue are most valuable when the material has been killed in corrosive sublimate, and are almost worthless where Hermann or any osmic acid reagents have been used. Sublimate is the killing reagent employed by Miss Foot, and from ova so treated she obtained the beautiful figures shown in her paper. As narrated above, sublimate shrinks and distorts the spindles in *Polychoerus* ova to such an extent that it has much less value as a reagent. Many animals killed in this way to study the vitelline glands proved to contain ova in which the first segmentation spindles were formed. The spindle fibers were often bent or distorted looking, but the general outline of the spindle was distinctly red and the periphery of the ovum equally distinctly blue. In no case have I found any substance taking the blue stain in the ovum which appeared to be archoplasmic or nucleolar in its origin.

In every individual, whether killed before, during, or after the breeding season, immature ova closely clustered near the lateral margins, quite close to the ventral wall of the body, are conspicuous in sections. The ovarian ova (Fig. 5) are, as a rule, oval in form, but are often packed so closely together that almost any outline is possible. The dividing cell walls are exceedingly indistinct and only to be made out in very well-stained sections. The ova are finely granular, and show so much greater affinity for almost all stains than do other tissues, that, in sections in which the ovarian ova are the special object of study, the color must be drawn until the other tissues are but slightly tinged.

Even the nuclei of mature ova stain less vividly than do those of immature cells. The probable reason for this is that at this stage the whole protoplasm which is to constitute a part of the future ovum is concentrated within the unripe ovum, and is undiluted by the food yolk, which stains less intensely, and

which has not yet been drawn from the vitelline gland. Dr. Sophie Pereyaslawzewa (3), in describing the young ovum of what she terms the "Pseudoacoela," — practically the equivalent of von Graff's Acoela, — speaks of fine grains of "vitellus nutritif" adhering to the surface of the nucleus. The fate of these grains she gives on page 149 as follows: "Cette disposition de toutes les parties inévitables de l'œuf reste telle jusqu'au moment de la fécundation, quand tout change: la vésicule disparait et emporte avec elle la force de l'attraction qui jusqu'à ce moment retenait les grains du jaune d'œuf adhérents à la surface de le vésicule; ils montent tous à la périphérie de l'œuf, y stationnent, participent dans cette position à tous les fractionnements de l'œuf et restent inséparables du vitellus formatif durant le développement embryonnaire. Comme il reste inséparable du vitellus formatif, il est difficile de préciser son rôle dans le développement embryonnaire." I have never observed either in the ova of *P. caudatus* or that of any other Turbellarian which I have studied any such changes as are here described.

The nucleus of the ovarian ovum is quite peculiar in its structure, and the change which it undergoes as the egg matures is worthy of some attention. In the smallest germ cell it is a clear structure with well-marked granular network extending from the nucleolus to the nuclear wall (Fig. 6). Within the nucleolus is a spherical spot which stains very deeply. This spot I have not been able to find in any but the ovarian ova. Immediately after the ovum passes into the vitellarium the whole nucleus increases enormously in size, so that its diameter is quite as great as that of the ovarian ovum. This increase is exceedingly rapid, for the intermediate stages are seldom found. Still more striking are the changes which the nucleolus undergoes (Fig. 7). Instead of a sphere, it grows to be an enormous coil of densely staining substance, which forms the most conspicuous feature in the whole ovum. The network of chromatin in the nucleus (Fig. 6) gives place (Fig. 7) to an exceedingly fine granular substance which stains less deeply, and in which but faint traces of the formerly well-marked network are to be seen.

In the stage shown in Fig. 7 the nucleolus has reached its maximum development, and from now on it decreases rapidly in size. As it decreases the structure of the whole nucleus changes. A network of granular substance (Fig. 8) resembling the chromatin granules of a still later stage occupies the whole nucleus, while occasionally fragments of this network stain as deeply as the chromatin granules of a later stage. For the most part, however, the affinity for color is much less than in the riper nucleus. The nucleolus has relatively diminished in size, but not in its staining qualities. It is to be noted that this structure never lies in the center of the nucleus, but always near the nucleolar wall. In Fig. 9, which represents the nucleus when fully ripe and ready to take part in the formation of the first maturation spindle, the nucleolus may be seen lying in close contact with the nuclear wall, completely broken up into fragments which are but faintly tinted by a stain for which in an earlier stage this structure exhibited the greatest affinity. On the other hand, the substance of the nucleus shows a distinct granular reticulum of chromatin particles which now for the first time stains deeply. In following the successive stages of the nucleus of the ovarian ovum (Figs. 5 and 6) through Figs. 7, 8, and 9, one cannot but be impressed with the fact that as the nucleolus diminishes in size and intensity of affinity for stain, the nucleus acquires these very qualities. This would suggest that the chromatin is built up at the expense of the nucleolus, rather than that the nucleolus is a by-product of the nucleus.

### *Formation of the Polar Bodies.*

Before entering on the description of the formation of these bodies it is necessary to make clear in what sense certain terms, which have been applied by different authors to different structures, are used in this paper. The organ which in this ovum presides over karyokinesis is a clear vesicle in which a dark staining spot is formed only after the amphiaster is well developed. In accordance with the terminology adopted by Wilson (13), I shall call this vesicle the centrosphere, and the spot

within it the centrosome. Also, a few words are necessary on the methods employed. Although in the egg-bearing animal, and without injury to the animal, it is easy to determine whether the ova are immature, about ripe, or whether the amphiaster of the first segmentation cleavage has already been formed, nothing further can be decided except by sections. That is, no information whatsoever in regard to the formation of the polar bodies or fertilization of the ovum can be obtained by examination of the ova within the living parent. It was, therefore, found most convenient and time-saving to kill large numbers of individuals which examination with a hand lens showed to contain large ova. Several of these were imbedded in one block and sectioned without attempting to orient the position of the ova. It was found that all the ova in one animal were in very nearly the same stage of development, but that the position of the axes of different ova differed; hence a section through the long axis of one ovum might cut the ova next to it at a very different angle.

In this manner many hundred animals were sectioned, each containing on an average half a dozen to a dozen or more eggs. The size of the ova allowed from six to eight sections through each, and as the whole worm was sectioned, the successive sections of any one ovum might be quite a distance apart. This involved the use of a low power in order to be sure that the section under inspection was the next in the series, and also every section had to be carefully examined with the highest power before the exact stage of development could be determined. Hence, the amount of labor necessary to obtain anything like a continuous history of the changes which occur in the ova has been very great.

It was found that by far the most frequent stages were either the nucleus intact, or the complete amphiaster of the first segmentation cleavage fully formed, showing that the changes between these two stages took place with great rapidity. There are, as will be pointed out later on, several short gaps in the chain of events between these two stages, but to fill these an indefinite amount of section cutting might be required, and the prospects of success were not sufficient to encourage

the undertaking. Also, it would have been desirable to have followed the history of the centrospheres and centrosomes into the segmenting ovum, but as related elsewhere (10), great difficulty was experienced in getting sections of ova after they had been inclosed in their gelatinous capsules. In nuclei in which the nucleolus had broken down and begun to disintegrate, I have noticed in two different cases, out of the many hundred examined, adhering to the inner side of the nuclear wall and in close proximity to the fragments of the nucleolus, a small, clear vesicle which strongly resembles the primitive archoplasmic vesicle as it appears in Fig. 9 outside of the nucleus. This would indicate an intra-nucleolar origin for this body. The substance of the nucleus in which this body is imbedded is, however, so dense and stains so deeply that it is impossible to be certain of this observation. The structure which I describe may or may not be the primitive centrosphere.

It is therefore possible that in this form, as in *Asteris* as described by Mathews (14), the centrosphere is developed and remains within the nucleus until the ovum is fully matured. The fact that at this time the nucleolus disintegrates suggests that the centrosphere may have been located within this dense mass. Although I have devoted much study to this point I have not been able to demonstrate whether the centrosphere actually migrates from within the nucleus or whether it originates in the cytoplasm.

I have sought in vain in young and almost mature ova for some trace of this body or of a centrosome. The most careful examination of serial sections, double stained with iron alum haematoxylin and with Bordeau red or orange G, fail to differentiate any such structure. If it exists in the cytoplasm, it must be exceedingly minute, or else does not at this stage respond to the same stains as it does later, and therefore must be of a different chemical structure.

When first seen the centrosphere is a small, dark-walled vesicle with a few short rays projecting in all directions (Fig. 9). The center of this vesicle is quite clear and stains red with Bordeau red or yellow with orange G. At this stage there is no centrosome or speck of any kind within the vesicle. Still

later this vesicle becomes dumb-bell-shaped and breaks into two bodies which (Fig. 10) for a time are connected by filaments. Apparently one of these bodies remains stationary while the other migrates through an arc of 180° to the other side of the nucleus, for, as Fig. 11 shows, one of the poles of the amphiaster is formed near the broken-down nucleolus, at which point the centrosphere first appeared.

In regard to the rays which extend out from these vesicles, it is difficult to say whether they are composed of the same substance as the walls of the vesicle, and therefore a part of it, or whether they are formed directly from the cytoplasm in which the centrosphere is imbedded. As, however, they grow in length as the vesicle increases in size, both the vesicle and rays must draw sustenance from the cytoplasm. These rays seem to grow at either outer end by a direct change of the cytoplasm, as Wilson (14) has described as occurring in the fibers about the sperm asters of *Toxopeneutes*.

As soon as the centrospheres have attained their positions at opposite sides of the nucleus the rays become speckled with numerous fine microsomes, and at the same time lengthen out so as to come in actual contact with the surface of the nucleus, which at those points becomes very irregular in contour (Fig. 11). It appears as if the ends of the fibers were exerting mechanical pressure on the surface of the nucleus and bending it in, but at the same time it is very evident that the nuclear membrane is being dissolved at these points. In a stage somewhat later than that shown in Fig. 11 the membrane is distinctly thinner, while in still later stages (Figs. 12 and 13) it has entirely disappeared, leaving no fragments in these regions, though it long continues round the rest of the nucleus. With the first disappearance of the membrane in this region, the substance of which the achromatic spindle fibers are to be formed appears. Apparently it is the linin of the nucleus which on the dissolution of the nuclear wall flows out toward the centrosphere (Figs. 12 and 13). At this stage it is homogeneous in appearance and does not show the fibrous structure which characterizes it later. It seems to flow directly out of the nucleus, and while so doing

to shorten the speckled rays from the centrospheres with which it comes in contact. In Fig. 11 it will be seen that these rays extend directly to the nuclear membrane; in Fig. 12 they are much shorter, while in Fig. 13 they have disappeared on the sides directed toward the nucleus.

As the achromatic fibers are thus being formed from the linin, that portion of the nucleus which lies directly between the two centrospheres undergoes differentiation. The particles of chromatin collect to form irregular clumps (Fig. 13) imbedded in clear linin. It looks as if the linin in flowing out toward the aster centers had brought the chromatin particles into contact with one another, and that these gradually melt together to form solid masses.

At first these masses show distinctly that they are made up of separate chromatin granules (Pl. XI, Fig. 29), but as the amphiaster develops the particles knit together, and as the achromatic rays begin to differentiate out of the amorphous linin, these clumps become elongated into irregular rod-shaped structures (Pl. X, Fig. 14, Pl. XII, Fig. 36). The number of clumps or rod-shaped masses formed is by no means constant. The manner in which the chromosomes are afterwards formed from them will be described later. As these rod-shaped masses are formed the whole amphiaster moves away from the broken nucleus which remains in the center of the ovum. Fig. 14 shows this process. From the various positions in which I have seen this amphiaster relative to the remnant of the nucleus, I am inclined to believe that one of the aster centers remains stationary as a pivot and that the other aster swings through a wide arc of nearly, if not quite, 180°, thus freeing the whole structure from the nucleus, and leaving the spindle free to move towards its destination, the surface of the ovum, which it does by moving very nearly in a straight line. The fact that one side of the nucleus is intact in outline (Fig. 14) while the other is completely destroyed bears out this view. Also, at this stage numerous fragments of the nucleus are scattered throughout the cytoplasm, particularly in the neighborhood of the broken side of the nucleus. When this is the case the whole cytoplasm stains more deeply than otherwise,

owing apparently to the stain-absorbing substance of the chromatin having been leached out into the cytoplasm. It frequently happens that sections through ova containing such fragments of the nucleus are perfectly unfit for study, owing to the deepness of the stain, while other ova in the same worm and on the same slide, and which, therefore, have been subjected to exactly the same treatment, are not at all overstained.

The larger portion of the nucleus as shown in Fig. 14 remains in about the center of the ovum and undergoes a rapid disintegration. The granular structure, in which the chromatin particles are so distinctly separated from the linin (Fig. 13), has completely disappeared, giving place to an ill-defined, muddy-looking mass, as if the stainable substance of the chromatin had leaked out and contaminated the hitherto clear linin as well as the cytoplasm. This must indicate a chemical change in both chromatin and linin. Also it should be noted that this muddy appearance extends out around the amphiaster, as if this structure while moving away from the degenerating nucleus had dragged with it some color-absorbing substance. The only chromatin which at this stage retains its former power of being sharply differentiated by stain are the clumps within the amphiaster, from which material the chromosomes are to be formed. While the amphiaster moves on its way to the surface of the ovum, the shattered portion of the nucleus gradually fades in distinctness, the cytoplasm around it still staining very deeply. Gradually, however, the nuclear substance is so completely assimilated by the cytoplasm that no trace of it remains, and the cytoplasm stains no more deeply than before the nuclear wall was ruptured. The relative quantity of substance taken from the nucleus to form the amphiaster as compared with the quantity assimilated or digested by the cytoplasm will be discussed later (p. 99).

While this amphiaster still adheres to the remnant of the nucleus, its length from aster to aster is of course greater than the diameter of the nucleus. When, however, it once breaks away from it, it is noticeable that instead of the asters drawing apart, they begin to draw toward one another. Thus the length shown in Fig. 14 is somewhat less than the diameter of the

nucleus. In Figs. 16 and 22 the length has evidently lessened considerably. It must either be that the substance of the spindle has undergone a condensation, or else that certain material has been dissolved into the cytoplasm. There is, however, no evidence as to how either of these changes may have been brought about. I have endeavored to determine the relation which the axis of the first maturation spindle, when first formed, bears to the axis of the first segmentation spindle, but with no satisfactory result. Fig. 26 is a diagram showing the dumb-bell-shaped form of the first segmentation spindle; AA the plane of the first cleavage; BB the plane of the second cleavage; P the polar bodies near the surface of the ovum. The plane AA divides the ovum into two equal macromeres. The plane BB cuts off two small cells which lie on the surface of the macromeres. The polar bodies always lie near the surface of one of the macromeres and near a point where AA and BB intersect. It is evident that if the first maturation spindle swung through an arc of 180° to break free from the nucleus remnant, and then traveled in a *straight* line to the egg surface, a line drawn through this amphiaster in anaphase when in contact with the egg membrane as shown in Figs. 16 and 21, and through the center of the ovum where the nucleus was, would give the axis on which the amphiaster was formed. Since, however, these facts cannot be accurately ascertained, except by studying whole transparent ova, the relationship of these axes still remains uncertain.

As the amphiaster moves towards its destination a very distinct and beautiful cytoplasmic network is formed, extending out from the asters. This could of course only be accomplished by the continual breaking down and building up of the cytoplasmic material; for while the whole structure is in motion there is at no time a distortion of the network (Fig. 22), such as would result by the movement of the structure through the ovum, — unless, indeed, the cytoplasm were very fluid and the network a rigid structure. That the amphiaster is much more rigid than the surrounding cytoplasm is shown by two instructive preparations which were the result of accident. Ova containing amphiasters in the stage now under discussion were

ruptured just before the worm containing them was placed in Hermann's fluid. The cytoplasm had flowed or been pressed out of the ovum carrying with it the amphiaster. In both cases the cytoplasmic network had been completely bent and twisted into a confused snarl. The achromatic rays were somewhat, but not nearly so much, distorted, but the centrospheres were almost unchanged. From this I infer that the amphiaster and the rays are on the whole much more rigid than the cytoplasmic network or the cytoplasm from which they were formed.

As one end of the amphiaster approaches the wall of the ovum the network is brought in contact with it, and as the amphiaster continues to approach, the network intervening disappears, being absorbed into the cytoplasm, although round the other aster the network continues as distinct as ever (Fig. 16). At no time does the network extend far into the cytoplasm, only the immediate neighborhood of the spindle being involved. The rest of the cytoplasm looks in no way different from that of an ovum in which the nucleus is still intact.

Shortly after the amphiaster has broken away from the remnant of the nucleus the centrospheres increase enormously in size, having a fairly reticular structure, merging gradually on the one hand into the chromatic rays which connect it with the cytoplasmic network, and on the other hand with the achromatic spindle fibers. Before the amphiaster is fully formed, indeed at about the time the linin of the nucleus is beginning to flow out (Fig. 12), an exceedingly small centrosome appears for the first time in each centrosphere, and as these structures enlarge the centrosomes become more conspicuous. By the time the amphiaster breaks away from the nucleus (Fig. 14), the centrosomes have become very prominent. In the stage shown in Fig. 22 I have been able to discern a small central black body within a small vesicle which stains a light blue. This, however, is difficult to demonstrate, for in order to distinguish these structures the stain must be of exactly the right intensity. The number of sections which show this stage is comparatively small, and many of them have been ruined — after being studied — in removing the cover slip and experimenting for the exact amount of stain necessary. I have not

been fortunate enough to find any stages intermediate between those shown in Figs. 16 and 22, therefore cannot detail the changes which the centrosphere may undergo; but as shown in Fig. 16, the centrospheres have become very much reduced in size, and the centrosomes have entirely disappeared. As shown in Fig. 21, a little more advanced stage, not only do the centrosomes disappear, but the centrospheres are finally reduced to small discs lying as it were on the ring of chromosomes. From this stage on, the cytoplasmic network fades until there is no trace of it left. The chromatic rays which connected the centrospheres with the cytoplasmic network are still left, though much reduced in size, as is shown in this last-mentioned figure. Fig. 17 shows the first polar body immediately after the division has taken place. A small cap of the substance which composed the central mass of the centrosphere rests on the ring of chromosomes, and below this may be seen the remnants of the achromatic fibers. At a later stage the fibers lose their structure and melt together with the stuff forming the cap, and in this mass the chromatin of the chromosomes collects in scattered particles (Figs. 19 *a* and 27).

The other daughter nucleus of the division lies close beside it (Fig. 19 *b*), and several times I have seen what I believe to be the first step towards a reorganization. The chromatin increases in quantity and breaks up into numerous fine grains, and at the same time the archoplasmic cap divides into two masses, Fig. 18, as if about to form the centrospheres of the new spindle. Fig. 19 *a* shows a polar body just formed, and *b* the other daughter product of the first polar spindle. In *b* the chromatin granules are gathered in what seems to be the region of the future equatorial plate. The achromatic rays are beginning to form, and at each pole clear but small centrospheres are formed. Between this stage and one in which there are two polar bodies side by side, and a third body, which without doubt is the female pronucleus, I have found nothing. The polar bodies remain side by side just within the egg membrane (Fig. 27) during several generations of cleavage cells and, as they become less conspicuous with time, probably they are ultimately absorbed.

Evidently the formation of the second body is accomplished with much greater rapidity than the first. Nor does this seem strange when it is borne in mind that the formation of the first maturation spindle involved action on a great nucleus, of which some parts are apparently selected and some rejected, and then the journey across half the diameter of the ovum, before the anaphase can be accomplished, while in the formation of the second polar body the substance involved is small and compact and no journey is necessary. Naturally, the process takes less time, and while I much regret that the full process of the reorganization has not come under my observation, the vast number of ova which one might have to section before finding the stages sought, presents too discouraging and uninteresting a piece of work to be contemplated.

In sections which show the two polar bodies side by side, and near them the female pronucleus, no trace of the archoplasmic cap is visible in the latter. The remnant of this body and of the achromatic fibers has entirely vanished. Instead we find a mulberry-like-looking object, composed of a large number of separate vesicles, each containing a round bullet-like granule of chromatin (Fig. 20). In this form it begins to migrate toward the center of the ovum, toward which the male pronucleus is also moving. During the passage it grows enormously and completely changes its structure. The collections of separate vesicles disappear and give place to a mass of chromatin grains imbedded in a linin network, almost indistinguishable except from its smaller size and absence of a nucleolus from the egg nucleus from which the first maturation spindle was formed. It is to be noted that the centrosomes in this form disappear with the anaphase.

### *Fertilization.*

In no section have I been so fortunate to find the sperm head in the act of entering the ovum, though I have frequently found it within the cytoplasm quite close to the surface. In such cases the outline of the ovum at the point nearest to the sperm head showed a marked protrusion. This presumably

indicates the existence of an "attraction" or "entrance cone," though the structure of this part of the ovum differs very slightly from the rest of it, except that it stains a little less readily. Hence the manner, as well as exact point of entrance with reference to the future plane of all cell cleavage, remains undetermined. The fact that, shortly after entrance has been effected, the sperm always lies near the pole opposite to that at which the polar globules are formed seems, however, to indicate that it has entered on the lower side of the ovum. At the time when the first maturation spindle begins to form, the sperm may generally be found at a distance from the surface of less than one-fourth the egg diameter, and the entrance cone has entirely disappeared. By this time the sperm has increased enormously in diameter and is surrounded by a peculiar substance (Fig. 23), which suggests in its appearance a ball or snarl of thread, and which stains but slightly. This substance must have been differentiated from the surrounding cytoplasm while the sperm is moving toward the egg center. While this growth of material about the sperm to form the complete male pronucleus takes place, that portion of the pronucleus which entered as sperm head increases very markedly in diameter (Figs. 24 and 25), and at the same time decreases in length as if it were melting away in the substance built up around it by itself.

As yet no rays or aster are formed in connection with it, although it progresses steadily toward the center of the ovum. This is of interest, for in some cases the movement through the cytoplasm has been attributed to the action of the aster and in others to the movement of the sperm itself. Since in this case no aster exists and the sperm is completely imbedded in a substance built up by itself from the cytoplasm and which moves with it, as if it were an integral part of it, the translation must be due to other forces. When close to the center of the ovum the remnant of the sperm head is represented by a crescent-shaped dark-staining mass (Fig. 25), still surrounded by a fibrous snarl of thread-like substance. Then the chromatin begins to increase by the breaking up of this head, until the whole pronucleus is filled

with it, and a more or less broken spireme is formed. The origin of the linin is difficult to account for, unless it is formed from the substance which has been drawn from the cytoplasm and which surrounds the sperm head (Figs. 23–25). At about this stage, at a point between the two pronuclei, but much nearer to the male than to the female, a small but distinct aster appears in the cytoplasm. The rays are very straight and clear, are not affected by the stain, and the central point shows no trace of a vesicle or centrosome. It can be described simply as the central starting point of these rays, and shows no structure which in any way differs from these rays. Subsequent stages show conclusively that this aster gives rise to the centrospheres of the cleavage spindle in which the large black-staining centrosomes lie.

It is, therefore, evident that here, as in the beginning of the first maturation spindle, the substance forming the centrosphere does not preëxist as such (unless too small to be seen), or that its chemical structure is so different that it will not react under the same stains as later. In regard to the origin of this aster it appears to be purely cytoplasmic. There is no evidence that it is in any way connected with the sperm nucleus. With the study of this point in view, hundreds of sections have been most carefully examined, but in no one could any distinct particle resembling a centrosome or vesicle be detected. A cytoplasmic origin of the centrosomes and centrosphere of the male pronucleus and consequently of the first cleavage spindle is too important a variation from the usually ascribed origin of these bodies to rest on anything but the best of evidence, and in this case the evidence is more of a negative than of a positive nature. It agrees, however, with the origin of the male centrosome as described by Wheeler (7) in *Mysostoma* and in *Allolobophora* by Miss Foot (12).

As this aster increases in size the chromatin in both pronuclei increases also, and except for the absence of nucleoli and their smaller size, either might be mistaken for the original germinal vesicle. The nuclear wall is, however, absent or but little developed. Soon the aster centers divide (Fig. 15), the rays from each extending out to the surfaces of the approaching

pronuclei, which they penetrate in a manner very different from that in which the aster rays of the first maturation centrosphere attach the egg nucleus, as will be explained presently.

### *Formation of the First Cleavage Spindle.*

The formation of this amphiaster is from its very inception so different from that of the first maturation spindle that the two can never be mistaken the one for the other. When in the first maturation spindle the two centrospheres begin to act on the nucleus they are at the opposite poles of the nucleus, while in the first segmentation spindle the centrospheres lie between the pronuclei (Fig. 15). I have never seen a complete union of the pronuclei until both have been deeply penetrated by the rays from the centrospheres. The relation of the rays to the chromatin granules is different in the two cases, as a comparison of Figs. 13 and 35 will show. In the former the rays which emanate from the centrosphere do not reach to the surface of the nucleus, while in the latter they pierce deeply into the chromatic substance, the granules of which seem attracted by the rays. In the first maturation spindle the centrospheres never draw widely apart so as to form a very large structure, while in the segmentation spindle the whole structure grows very rapidly and soon occupies the whole interior of the ovum. In the former the clumps and rods of chromatin are formed before the spindle breaks away from the nucleus, while in the latter these rods and clumps are first formed when the spindle has almost attained its full size (Figs. 36 and 37). It is evident, however, in both cases that the bulk of the chromatin contained in these clumps far exceeds the amount which at a later stage is found in the chromosomes. It is clear, therefore, that either a condensation of particles takes place or else that some of the material in the clumps is removed. That this latter is the case is very clearly shown in a later stage before the chromosomes are completely formed. But before the description of this is given it will be well to account for that portion of the pronuclei not drawn into the clumps.

As the centrospheres draw apart, the nuclei break to pieces and the whole substance, except what is contained in the clumps, is scattered throughout the cytoplasm, which in consequence stains much more deeply for a time. Meantime, when this spindle has about attained its full size the rods of chromatin do not continue to elongate, but on the contrary flow together at about their middle points, thus forming a continuous ring which lies in exactly the position afterwards occupied by the equatorial plate. This ring even while forming has numerous outward prolongations extending into the surrounding cytoplasm (Figs. 28 and 30). Sections showing this structure were very carefully studied, for nothing similar to it has to my knowledge been described in amphiaster formation. To guard against the possibility that this structure might be an artifact, ova killed in Hermann's, corrosive, corrosive acetic, Flemming's, formaline, and picro formaline, were sectioned, and the structure as here figured was found in ova killed with all these different reagents; therefore it can be stated with confidence that it occurs normally in the formation of the amphiaster in this egg. As is shown in Fig. 28, the prolongations, or equatorial rays as they might be called, are composed for the most part of separate granules of chromatin, while the chromatin which occupies the position of the future chromosomes is much more compact. This difference I believe to be due to the absorbing action of the cytoplasm on these exposed rays. It may indicate that as the granules aggregate to form the chromosomes, the surplus material flows away in the form of these equatorial rays. The number of rays is apparently the same as the number of chromosomes. At a little older stage, sections through the equatorial plate (Fig. 34) show thirty-one cheveron-shaped chromosomes, from the bases of which numerous dark anastomosing lines radiate outward. These apparently are in a measure the remnants of the chromatin prolongations and the whole area tinges somewhat more deeply than the rest of the cytoplasm, exactly as is shown in Fig. 14, when chromatin is dissolved by the cytoplasm. Later the cheveron-shaped chromosomes break at the apexes to split into two.

The manner in which the chromosomes were formed in the first maturation spindle seems to be exactly the same as here described, but the parts involved are so much smaller that it is more difficult to make them out; also, since the duration of time from the entrance of the sperm to the complete formation of the first maturation spindle is very short, the number of sections obtained through the polar spindles are but few. In no case did I obtain a section directly in the plane of the equatorial plate during its formation, but several diagonal sections show clearly that a structure similar to that here described is formed in the maturation spindle. The true significance of this reduction of the quantity of the chromatin is difficult to explain. Furthermore, the reduction occurs twice during the formation of each spindle. First the clumps of chromatin are selected from the nuclei and then but a portion of these clumps are taken to form the chromosomes. There are very many cases recorded in which but a small portion of the nucleus is utilized, and the rest dissolved into the cytoplasm, but in this ovum the quantity seems unusually great.

I have endeavored to form some estimate of the relative quantity in the chromosomes as compared with the bulk of the nucleus, by taking a small apple about the diameter of the magnified nucleus as shown in Fig. 7, and dividing it in halves, quarters, eighths, sixteenths, etc. The seventh division, which would be $\frac{1}{128}$ of the whole, would certainly afford many times more material than is contained in all of the chromosomes in the cleavage spindle, and a comparison of Figs. 22 and 34 shows that the material in the chromosomes of this spindle is vastly more bulky than in the polar spindle. Since it is thus clear that a very small fraction —possibly not more than $\frac{1}{500}$— of the chromatin substance of the germinal vesicle and later of the pronuclei is preserved as chromosomes, while the rest is dissolved, the conclusion is immediately forced on us either that the chromatin of the chromosomes differs chemically from the bulk of the chromatin or that the chromosomes are protected mechanically from the dissolving properties in cytoplasm. When it is remembered that the chromatin of the polar bodies (Fig. 27) remains intact for many cell generations

in the very substance which so quickly dissolves and assimilates the rest of the nucleus, there seems no escape from one or the other of the above conclusions.

Again, in the cleavage spindle the chromosomes remain practically unaltered for days (p. 77), while the enormous nuclear chromatin once scattered through the cytoplasm dissolves in a very short time. We cannot, therefore, but conclude that they must differ chemically, or else that the achromatic rays or some other substance builds around the chromosomes a wall protecting them from the attacks of the dissolving agents in the cytoplasm. The greater portion of the clumps or rods of chromatin described above must have the same structure as the bulk of the nucleus, for they also are dissolved, though more slowly, and only the chromosomes are left. Since it thus appears that the substance of the chromosomes differs from that of the rest of the chromatin of the nucleus in being insoluble, there are three possibilities presented as to the manner in which this difference may have arisen: — *First:* that two distinct chromatic substances have existed prior to the spindle formation, one soluble and the other insoluble; the former destined to be formed into chromosomes bearing the hereditary traits, and the other, food for the cytoplasm. *Second:* that there is but one chromatic substance in the nucleus, and that this is soluble in the cytoplasm; as the spindle is formed certain particles are changed into insoluble stuff from which the chromosomes are formed; with the breaking down of the nuclear wall the rest of the chromatin is exposed to and dissolved by the cytoplasm. *Third:* there is but one chromatic substance in the nucleus and that this substance is insoluble in the cytoplasm; of this substance the chromosomes are formed; the rest undergoes chemical degeneration and becomes soluble in the cytoplasm.

Now to consider these three propositions in the order stated above. If we accept the first, we must assume that some force causes a migration of the insoluble rather than the soluble particles toward the center of the nucleus to be formed into clumps. This migration, however, if it occur, does not visibly disturb the structure of the rest of the nucleus. On

the other hand, the fact that the greater portion of these clumps is dissolved when in the form of equatorial rays, might be explained by supposing that the condensation of the already formed chromosome particles drags with it granules of the less differentiated, soluble chromatin which have in them nothing which is to be transmitted to the next cell generation. This theory in no way conflicts with the "reduction theory" or with the existence and transmission of the "determinants," "ids," etc., of Weismann. It simply asserts the existence of two chromatic substances in the nucleus, which, though microscopically indistinguishable, differ in the fact that the one which is retained contains all that is essential to heredity, while the other contains substances which are not to be transmitted to the daughter nuclei, but become a part of the cytoplasm.

The absorption by the cytoplasm of some of the products of the nucleus is not an uncommon phenomenon, and the connection between the nucleus and yolk nucleus (15) shows that chromatin is not necessarily a substance transmittible to the chromosomes alone. An objection to this theory is that it assumes the existence of a force to hold together or to collect the chromosome particles containing the "determinants" and "ids" near the center of the nucleus. If the second proposition is accepted, we must assume that the insoluble particles received from the parent oögonia have degenerated, for they were at one time insoluble; if the third is accepted we must assume that the greater portion of the hereditary qualities existing in the nucleus are dissolved up as food for the cytoplasm. The supposition then that there are two kinds of chromatin stuff, the one insoluble and bearing the heredity which is to be transmitted to the daughter cells, and the other food for the cytoplasm, seems unavoidable.

The exact use of this food is of course pure conjecture, but the following suggestion may be worthy of notice. When the soluble chromatin is thrown out into the cytoplasm, it is digested quite rapidly; and then, and not till then, do the centrospheres (which up to this time have been very small) begin to grow and attain their full size. May it not be that the

soluble chromatin affords to the cytoplasm the material necessary to supply this growth?

To return now to the growth of the first segmentation spindle. By the time the chromatin has become condensed into clumps the achromatic fibers have become much more distinct. As stated above, the rays from the aster centers seem to grow down into the pronuclei (Fig. 35). This is a marked contrast to what appears to take place in the formation of the first maturation spindle. In this latter, when the nuclear wall is dissolved the linin appears to flow out and form the achromatic rays; while in development of the segmentation spindle the rays from the aster centers appear to grow down into the pronuclei. It is of course possible that the linin into which they grow affords material for that growth, but since the bulk of these achromatic fibers soon far exceeds that of the linin, it is evident that material is being elaborated from other sources to form these rays. In the fully formed spindle (Fig. 37) these rays are exceedingly large and distinct.

As these fibers develop, an exceedingly strong cytoplasmic network surrounds the whole spindle (Figs. 36 and 37). This network is dotted with numerous microsomes which with iron haematoxylin stain a deep blue, not unlike the centrosomes. This network soon extends to the very uttermost limits of the ovum, so that the first superficial section, which may cut off but a very small portion of the ovum, reveals the network and fully prepares the observer for the large spindle which deeper sections will disclose. As the network thus extends further into the cytoplasm, the rays supporting it and connecting it with the aster centers increase in size and length. In a former paper (10) mention was made of the strange manner in which certain pigment granules are moved about so as to lie in the same plane as the equatorial plate, and reference (16) to similar observations in other ova is made. The size of the rays and network in the ova is shown in Fig. 37 and also in Fig. 28, where the polar globules are surrounded by the network.

At the time when (10) was written I had sectioned but few well-preserved ova and was unacquainted with the remarkable structure of this spindle, but while examining certain pigment

granules which are very characteristic of these ova I was struck with their peculiar movements on the surface of the egg. To quote the description written at the time: "Not infrequently while examining the surface of a (living) ovum with an oil immersion lens, I have seen one of these granules come up from within the ovum and move across the field of vision. . . . When the ovum is thus viewed it is clearly suggested that there are wonderfully active forces at work within, for the surface fairly scintillates with the movements of the protoplasm and these pigment granules." Had I at that time known the size of the spindle rays and the extent of the cytoplasmic network, I should have felt less wonderment at the strange movements of the pigment granules. These always find final lodgment in a ring over the equatorial plate when the cytoplasmic rays are least developed. While watching the surface of an ovum in the two-cell stage I have seen one of the polar bodies lying close inside the egg membrane bulge out the surface almost to the bursting of the egg membrane, as if by pressure from below. In the paper (10) on the segmentation of this ovum the peculiar distortions of the ovum in different stages are referred to and figured. I cannot but believe that much of this is due to the movements of cytoplasmic rays.

At the stage shown in Fig. 36, when this remarkable cytoplasmic network was beginning to form, the centrospheres can hardly be said to exist as a distinct structure, for when the cytoplasmic network ceases the achromatic fibers begin. There are, however, small clear spaces at the points from which the achromatic fibers radiate. These spaces are the beginnings of the centrospheres. As, however, the spindle grows, the centrospheres enlarge very rapidly and become most conspicuous structures. They at first are clear, colorless structures, but as maturity approaches they become somewhat granular, with occasional dark specks scattered here and there. Soon in the center of each appears a clear, translucent spot in which there is a small but distinct black or dark blue staining centrosome. If the section is at all over-stained, the centreole will stain as deeply as the centrosome, so that the whole structure, both centreole and centrosome, appears like an enormous

centrosome, while the other portions of the amphiaster are not markedly over-stained. When, however, the color is properly drawn the centreole is tinged a faint blue, as is shown in Fig. 36. There are at this stage a few faint, dark fibers which radiate into the surrounding centrosphere from the centreole. By the time the chromosomes are fully formed, these radiations are exceedingly distinct (Fig. 31), but from this stage on they begin to fade away. Also as the radiations fade the centrosome enlarges so as almost to fill the centreole, and at the same time it elongates in a direction at right angles to the long axis of the spindle. This elongation occurs at about the time the equatorial plate is completely formed, Figs. 31 and 34 being portions of the same amphiaster. I believe this is the stage at which the ova are normally laid, for the anaphase does not occur until after oviposition.

It is, however, not unusual to find a centrosome, as shown in Fig. 32, apparently consisting of numerous fine granules which stain very deeply; also where it is exceedingly indistinct, as in Fig. 33, although I have found no sections through fully formed spindles when it is altogether absent. It is noticeable that in these cases the whole spindle is somewhat indistinct in outline, and I believe is undergoing the retrograde development described in p. 77. The large number of cases cited by Wilson (13) and others, in which the centrosome is shown to be a permanent organ of the cell, presents a curious contrast to its action in this ovum. The small body shown in Fig. 9, which for want of a better name I have called the archoplasmic vesicle, may be permanent, but the spot within it, the centrosome, is not, unless its chemistry so changes that it answers to a stain at one time and not at another. It should be borne in mind that these studies are made on different series hardened in sublimate, sublimo-acetic, Hermann's and Flemming's fluids, and in no case have I found any trace of a centrosome except when the spindle is well advanced in its formation.

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## DESCRIPTION OF PLATE IX.

All figures on this plate except Figs. 1, 2, 4, and 5 are magnified about 1140 times.

FIG. 1. Diagram showing relative position of ovary and vitellarium; *o.*, ripe or nearly ripe ova in the vitellarium; *ov.*, ovary; *vt.*, vitellus.

FIG. 2. Immature ova in the vitelline gland; *o.*, ova.

FIG. 3. Vitelline gland cells; *n.*, nucleus.

FIG. 4. Ripe ovum gorged with material from the vitellus.

FIG. 5. Ovarian ova.

FIG. 6. An ovarian ovum; *n.*, nucleus; *nc.*, nucleolus.

FIG. 7. The nucleus of an ovum after it has just passed into the vitelline gland; *nc.*, nucleolus.

FIG. 8. The nucleus of a nearly ripe ovum; *nc.*, nucleolus.

FIG. 9. Nucleus with the archoplasmic body when it first appears; *ap.*, archoplasm.

FIG. 10. Division of the archoplasmic bodies.

FIG. 11. Beginning of the first maturation spindle.

FIG. 12. Same.

FIG. 13. Same.

## DESCRIPTION OF PLATE X.

All figures on this plate except Fig. 26 are enlarged 1140 diameters.

FIG. 14. First polar spindle leaving the nucleus.

FIG. 15. Sperm aster forming between the pronuclei.

FIG. 16. First polar spindle near the surface of the ovum.

FIG. 17. First polar body just formed.

FIG. 18. Regenerating nucleus after the first polar has been formed.

FIG. 19. (*a*) First polar body. (*b*) Regenerating nucleus.

FIG. 20. Female pronucleus regenerating after formation of the second polar body.

FIG. 21. Anaphase of first polar spindle.

FIG. 22. First polar spindle showing centrosomes. (The boundaries of the asters are drawn distinctly. No such lines exist in nature.)

FIG. 23. Spermatozoon shortly after it has entered the ovum.

FIG. 24. Spermatozoon somewhat nearer the center of the ovum.

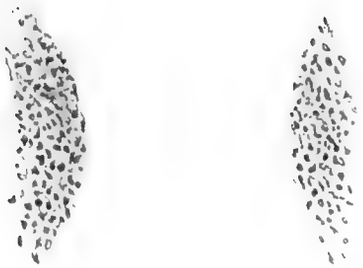
FIG. 25. Spermatozoon still nearer the center of the ovum.

FIG. 26. Diagram of ovum in which the first cleavage spindle appears like a translucent dumb-bell-shaped structure; *P*, the two polar bodies; *AA*, first plane of cleavage; *BB*, line of second plane of cleavage.

FIG. 27. Polar bodies in the cytoplasmic network of first cleavage spindle.

16

17





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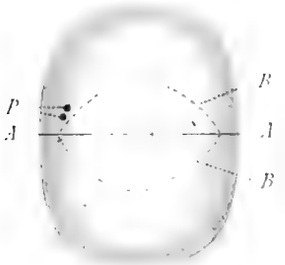



27.

## DESCRIPTION OF PLATE XI.

FIGS. 28, 34, and 35 magnified 1140 times, all others 1500 times.

FIG. 28. Section through the region of the equatorial plate before the chromosomes are formed.

FIG. 29. Particles of chromatin consolidating in the nucleus.

FIG. 30. A portion of Fig. 28 enlarged.

FIG. 31. Centrosome.

FIG. 32. Centrosome.

FIG. 33. Centrosome.

FIG. 34. The equatorial plate.

FIG. 35. A portion of the sperm aster and the male pronucleus.

## DESCRIPTION OF PLATE XII.

Both figures enlarged 1500 diameters.

FIG. 36. Beginning of the first cleavage spindle.

FIG. 37. One quarter of the first cleavage spindle.

56.

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## THE OVARIAN EGG OF LIMULUS.

*A CONTRIBUTION TO THE PROBLEM OF THE CENTROSOME AND YOLK-NUCLEUS.*

JOHN P. MUNSON.

TABLE OF CONTENTS.

## INTRODUCTION.

THIS work on the history and morphology of the ovarian egg of the King Crab — *Limulus polyphemus* — has been done in the Zoölogical Laboratory of the University of Chicago, and in the Marine Biological Laboratory at Woods Holl, Mass., under the direction of Professor Whitman.

The object of the work has been to determine, so far as possible, the organization of the egg during its different stages of growth, and to give a connected history of its phases. The vitelline-body and centrosome have received special attention.

Much of the more valuable literature on this subject is of so recent date that it has not seemed advisable to encumber the paper with a historical compilation. Papers of special interest will be referred to in connection with my own observations.

*Historical.* — The main facts concerning the position and external form of the ovary have been known since 1828, when Strauss Durckheim made known the internal anatomy of Limulus.

Somewhat later, 1838, J. Van Der Hoeven also published an account of the ovary, its mode of branching, its ramifications throughout the cephalothorax, and the astonishing number of eggs produced.

Lockwood ('70) has treated in a popular, but exceedingly interesting way the habits of Limulus, and mentions many interesting facts in regard to its development.

Packard ('71) noted some points in the development of the ovary, and among other things called attention to the laminated structure of the egg membrane, which he calls a chorion.

Owen ('73) described and figured the ovary of Limulus, and showed the relation of the ovarian tubes to the terminal oviducts, as well as the relation of the right and left ovary to each other and to the underlying parts.

Among those who have devoted attention more directly to the nature of the ovarian eggs may be mentioned Ludwig, Gegenbaur, and Kingsley.

Ludwig ('74) called attention to the character of the germinal epithelium, and especially to the cell nature of the egg. He, however, based all of his conclusions on the observations

of Gegenbaur ('58), who, he says, has had the good fortune of having a living specimen for dissection.

Kingsley ('92) has shown the relation of the egg to the germinal epithelium, and, from the point of view of oögenesis, has shown some of the similarities of Limulus to the spiders.

No one, however, so far as I know, has attempted to study the ovarian egg of Limulus with the more fundamental problems in view. I have been compelled to go over the whole ground and to reëxamine the observations previously recorded concerning the ovary. Where my description agrees with previous accounts, it has at least the value of a confirmation.

*Material.* — Material for the study of the mature eggs was obtained, through the kindness of Professor Whitman, from three female specimens that had been on exhibition in the aquaria of the United States Fish Commission at the World's Columbian Exposition in Chicago. In the following June, July, and August an ample supply of material was collected at Woods Holl, Mass., consisting chiefly of material from females having mature ovaries, and captured in the act of ovipositing. From some of this material, through natural and artificial fertilization, a large number of embryos were produced and raised to the desired age and size. Young Limuli, ranging from one-fourth inch to eight inches, were obtained in abundance at North Falmouth, Mass.

The results here presented have been confirmed and extended on material ranging in size from eight inches to the adult form, obtained in the latter part of October and the first week in November, off New Haven, in Long Island Sound, in water ranging from five to fifteen fathoms. The material has been very abundant and the series complete. My thanks for material are due to the following gentlemen: W. H. Munson, W. H. Packard, Dr. Watasé, and Professor Whitman. I would also acknowledge my obligations to Captain Barnes, of the Oyster Steamer, Roe & Co., of New Haven, Conn.

My former teacher, Prof. Sidney I. Smith, has very kindly enabled me to use the Yale Library; and Dr. Watasé has given me much encouragement in my work. I desire to express my appreciation of these favors.

*Methods.* — The best preserved material of the young forms was obtained by killing in (1) Kleinenberg's picro-sulphuric, (2) corrosive-acetic, and in (3) a mixture, in equal parts, of a ten per cent solution of nitric acid and picro-sulphuric. Material killed in this latter mixture was excellently preserved. It has the advantages of staining readily, and is especially suited to the double stain of Lyon's blue and lithium-carmine. By this means, the archoplasm and centrosomes are made very distinct. It was not so favorable for the study of the various phases of karyokinesis. For this, material preserved in Merkel's fluid was used.

The ovaries of the adult animal successfully preserved in (4) Merkel's fluid are excellent for the study of the centrosomes and sphere. This fluid, however, does not always give equally good results, even when most carefully applied. Slight irregularities in the preparation of the mixture, as well as differences in temperature, may account for some of the differences in the effect; but physiological variations in the egg itself, especially those changes arising from a constantly increasing quantity of food material, are perhaps responsible for much of the variation.

In that stage of the egg immediately preceding its escape from the follicle, the following method was successfully employed: (5) one-fourth per cent aqueous solution of platinum chloride applied for twenty-four to forty-eight hours, and the eggs then passed through the various grades of alcohol. The eggs may also first be killed by leaving them a few minutes in Flemming's fluid and then transferring them to platinum chloride for forty-eight hours.

For the stages of the mature egg, after it gets into the ovarian tube, Kleinenberg's picro-sulphuric has been found most favorable, where attempts to imbed in paraffine have been made.

Owing to the difficulty of staining after Flemming's and Hermann's fluid, these have not been extensively used, although a number of the drawings have been made from preparations of material preserved in this way, as well as from material hardened in corrosive sublimate, corrosive-acetic, and picro-sulphuric.

Imbedding has been done in the usual way by means of paraffine. The mature eggs of the ovarian tubes were imbedded and sectioned in celloidin. To enable the imbedding medium to penetrate, a slit was made in the chorion by means of a sharp razor.

Previous to imbedding, the absolute alcohol was removed by means of chloroform, saturated with dissolved paraffine. To avoid the hardening effect of the chloroform on the yolk of the larger eggs, xylol and turpentine were substituted for the chloroform.

The paraffine sections, from five to ten $\mu$ in thickness, were fixed to the slide by means of water, Mayer's albumen fixative, or by the two combined.

Staining was done almost exclusively after the sections were mounted on the slide. The sections of the larger eggs in celloidin were stained with Delafield's haematoxylin diluted ten times with water and slightly acidulated with HCl. This leaves the yolk spheres unstained and facilitates the search for traces of the nucleus and the maturation spindle.

For the study of karyokinesis, Heidenhain's iron-haematoxylin was used. The archoplasm and centrosome in the younger eggs were studied by means of Heidenhain's iron-haematoxylin, either alone or followed with erythrosin, eosin, or acid fuchsin. Erythrosin and cyanin have also been used to good advantage; also borax-carmine, followed with picric acid; Delafield's haematoxylin, either alone or followed with picric acid; Weigert's picro-carmine; Ehrlich's haematoxylin, either alone or followed with erythrosin, eosin, and especially with acid fuchsin; eosin and nigrosin to a limited extent; the Biondi-Ehrlich mixture, and finally lithium-carmine and Lyon's blue.

These stains all give good results, but they differ in the extent to which they can be applied. The carmine stains have not been found useful on material killed in Merkel's or Flemming's fluids. To obtain the double stain with Lyon's blue, safranin has been substituted for the carmine on Merkel's material. In such cases the sections were first stained in Lyon's blue for twenty-four hours, after which they were stained for twenty-four hours in safranin; and previous to

clearing in xylol, the sections were dehydrated in absolute alcohol, containing powdered copper sulphate. This method was reversed when lithium-carmine could be associated with the Lyon's blue. With the method above described, I have not found it necessary to watch the stain under the microscope with the care which Miss Foot ('96) seems to think is necessary. On the whole, Ehrlich's haematoxylin, followed with acid fuchsin, has been the method that I have placed greatest reliance on in the case of material killed in Merkel's fluid. Weigert's picro-carmine has also been found very useful. The specific effect of each is more profitably stated in connection with the description which follows.

It has been found very profitable to verify as many of the points as possible on the living material. Much uncertainty has been removed in that way.

## OECOLOGY.

*Oviposition.* — Many of the observations of Packard ('70), Kingsley ('92), Lockwood ('70), Agassiz ('78), and others have been confirmed. Oviposition, at Woods Holl, takes place during the months of May, June, and July. The females at this season frequent a particular beach characterized by an abundance of medium-sized sand and the entire absence of rocks. They appear to come in with the tide. As stated by Kingsley and others, they are usually accompanied by one or more males, one of which has attached himself to the posterior margin of the female carapace, the other males occupying a similar position with reference to him and to each other.

If the male occupying the position described be seized and raised out of the water, he does not let go his hold, but lifts the much larger female out with him. If they are then dropped into the water, they continue the same gait as if they had never been molested.

The attachment of the male appears to take place in deeper water; but frequently isolated males may be seen moving over the shallow bottom off shore, apparently in search of females, which, when they approach, they appear to recognize at consid-

erable distances. Isolated females are also met with; but oviposition in the absence of the male was not observed.

I can confirm Lockwood's observation to the effect that they deposit their eggs at the point reached by the highest tides. But Kingsley is also right when he affirms that there are exceptions to this rule. Thus I have found them ovipositing at a point where I was in some doubt as to whether the eggs would ever be exposed to direct rays of the sun. On the other hand, the nests can be found at the point reached by the high tides, even where no superficial evidence of their presence is visible, — an evidence that they are numerous at that point. During oviposition the animals may be covered with as much as a foot or more of water; but they usually approach so near shore that their carapace is only partly covered.

The act of ovipositing is apparently accompanied with considerable activity and excitement, which is indicated by an accumulation of air bubbles on the surface of the water, forming a distinct line, extending in the direction of movement of the animals. By pursuing this line, they can be traced for considerable distances. In ovipositing, the female is partly buried in the sand, and only slight movements are visible from above; but the appendages are evidently in rapid motion, excavating a deep cavity from which the finer sand becomes sifted out, and into which the eggs are discharged. The eggs thus come to lie in the midst of sand peculiarly resembling the eggs both in size and appearance.

Careful examination of these nests would seem to indicate that the terminal oviducts are discharged into each nest.

It seems probable that most, but not all, of the eggs contained in the ovarian tubes are laid during one season. The females captured on June 1 usually show a turgid condition of the ovary. At the end of the laying season, on the other hand, the ovarian tubes are nearly collapsed.

On the other hand, there is much evidence to show that a female Limulus does not oviposit every year, and that females having the ovarian tubes filled with eggs may, even in a state of nature, carry these over at least one season. On the twenty-fifth of October, in Long Island Sound, females were

taken by me whose ovaries were turgid with what appeared to be mature eggs, and which could not be distinguished from those examined at Woods Holl at the beginning of the spawning season. Others presented ovaries in which only comparatively few eggs had arrived in the ovarian tubes. The condition of ovaries taken from the females that had been on exhibition at the World's Fair was that of the former, *i.e.*, the ovarian tubes were filled with mature eggs. This being on November 1, it was concluded that the eggs had been retained because of the confinement of the animals during the season when oviposition takes place. The above observation shows that, in their native element, females with ovaries in a similar condition, at the same season of the year, are abundant, and that neither the fullness of the ovarian tubes, nor the apparent maturity of the eggs, are sure indications of the time when oviposition will take place. Yet, as Kingsley has observed, there are reasons for believing that there is no oviposition in confinement. The ovaries of the female Limuli kept in the large floating aquaria of the Marine Biological Laboratory at Woods Holl were always filled with mature eggs, even after the spawning season was over.

The movement of Limulus is a uniform, gliding one. Oblivious of everything except the business which occasions its visit, it pursues a more or less direct path for the beach, which is most favorable to the concealment and subsequent development of its eggs. Any attempt at concealment or betrayal of fear, by a hasty retreat, is not to be observed. If, on its first arrival off the beach, it be disturbed, it cannot be induced to deposit its eggs, but endeavors stubbornly to make its way back into deep water. The male still clings to the female, but I have in vain, for hours, endeavored to secure freshly laid eggs by urging them towards the favorable point of oviposition.

*Moulting.* — Lockwood found a soft-shelled specimen in the month of February, and concluded that, while the young moult four or five times the first year and adults usually only once, in the month of August, there might be two moults a year even in the case of the adult. As several soft-shelled ones were

observed by me on the twenty-eighth day of October and the first of November, it may be supposed that the moulting period is not fixed to definite seasons, but that it may take place at any time, according to the physiological condition of the animal.

Although the soft-shelled females observed by me were of a size not inferior to the largest hard-shelled specimens found to possess an ovary filled with mature eggs, yet the eggs in these soft-shelled ones were scarcely visible to the naked eye. The larger eggs, however, when exposed to favorable light, showed a decided reddish-pink coloration, indicating the second stage of yolk formation. Numerous specimens, apparently fully grown, appeared to have moulted at an earlier period. These were distinguished from the hard-shelled ones, having mature eggs, by the translucency of the carapace, and their consequent brown appearance in contrast to the black appearance of the hard-shelled ones. The carapace in the former was further distinguished by many distinct internal markings not visible in the hard shells; and, unlike the latter, the eggs had not yet been discharged into the ovarian tubes, but many of them, apparently, had reached follicular maturity. In no case did I find one recently moulted containing mature eggs. All females that were observed ovipositing at Woods Holl had hard shells.

*Habitat.* — Limuli, ranging from eight inches to the adult forms, were found in abundance on the last of October and the first of November, off New Haven, in Long Island Sound, in water ranging from five to fifteen fathoms. It was the general opinion among the oyster fishermen, who are engaged in dredging oysters during the greater part of the winter months, that Limulus goes into deeper water later in the season. But very little reliance can be placed on their observations; for, although they had been engaged in dredging starfish previous to November 1, yet they seemed to be ignorant of the presence of Limulus at that time, till they were asked to take notice of them.

The earlier stages of Limulus, ranging from one-fourth inch to eight inches, were abundant at North Falmouth, Mass., in the month of August. The place where they are found is a

large, level expanse of loose sand that is left entirely exposed on the retreat of the tides, and receives fresh water from a stream flowing into the estuary, as well as from several fresh-water springs along the shore. The sand is rich in clam shells and soft-bodied animals, and the abundance of organic material is evidenced by the black coloration of the sand in which the young Limuli live.

At low tide they lie quietly buried, just below the surface, and no tracks or markings reveal their presence. As soon as the incoming tide has covered the sand, however, the Limuli begin to move about, not on the surface of the sand, but just beneath the surface, being always covered with the uppermost layer of sand. This upper layer has the usual color of sand, while just below it is black. As the little Limuli plow their way along, the upper layer is pushed aside and a black track appears. Immediately after the incoming tide covers the sand, these black lines appear running in every direction. The beginning of the black line marks the resting place of the little Limulus during the absence of the tide; the end marks the distance which it has traveled. At this point it can always be found. The search for these little creatures is, therefore, a comparatively easy one, notwithstanding their protective coloring and their subterranean mode of locomotion. This mode of locomotion is evidently useful to them as a means of protection from the many enemies that infest the neighboring eelgrass. Many little Limuli, departing from the path which nature has marked out for them, can be seen to have fallen victims to these enemies.

*Food.* — One large female Limulus was found nearly buried in the mud in about three feet of water. An examination showed that it was enjoying its dinner, which consisted of a worm.

In Dr. Lockwood's vivid description of the habits of Limulus, an instance is cited where it had been observed caught by a clam, which, it was supposed, the Limulus had been trying to consume. From the oyster growers at New Haven, and especially from Captain Barnes and his crew, it was learned that, while the starfish is their dreaded foe, the horse-fish (which is

the fisherman's name for Limulus) is perfectly harmless so far as oysters are concerned. I was delighted, however, after traveling from Chicago to New Haven in order to secure material late in the season, to find that they were abundant on these oyster beds.

*Vitality of the eggs.* — At Woods Holl, in the summer of 1894, the eggs, laid while the animals were observed in the act, were taken from the nest as soon as the animals were about to leave. The animals were also taken. Owing to the secretion of the oviduct covering the eggs, they adhered more or less firmly to the sand, with which they were intimately mixed, and formed balls. These eggs and sand were put into a dish, No. 1, containing sea-water. A considerable quantity of eggs were taken from the ovary of the same female, and placed in a dish, No. 2; and, after treating them with the contents of the male genital ducts, the dish was rotated; the eggs arranged themselves in a single layer, and adhered firmly to the wall of the dish. In the bottom of another dish, No. 3, were placed a number of glass slides, and eggs from the same source similarly treated. They were fixed to the slides in the same way. In a dish, No. 4, eggs and sand from another nest were placed, and, like the others, supplied with sea-water. Dish No. 1 was moved only sufficiently to change the sea-water occasionally. Dish No. 2 was treated in the same way. The glass slides in No. 3, with the eggs adhering, rested on supports, and were frequently turned, so that the eggs were alternately above and below the slide. The sand and eggs in dish No. 4 were vigorously stirred several times a day. In all four dishes the eggs developed. At the end of two months no perceptible difference, so far as the development of the eggs was concerned, could be observed. By means of the glass slides, the experiment of Patten ('94) was confirmed; but the changes in the first indications of cleavage observed by him have no perceptible influence on the development of the eggs.

The above experiments were begun on June 25. The eggs were not exposed to sunlight, and the development was slow and irregular. On the first of September many of the embryos had hatched, while many of the eggs showed no sign of devel-

opment, and indeed appeared as if they were more or less decayed. Embryos, however, continued to be produced, and the eggs and sand were removed to Chicago in jars, where the development continued, the evaporated salt water being replaced from time to time with ordinary fresh tap water. After preserving as many of the different stages as seemed desirable and no more embryos being hatched, the jars were put to one side and neglected till a few days before Christmas, when a hasty examination showed that the water had evaporated and all signs of life had disappeared. In order to clean the jars they were filled with tap water and allowed to soak over night. The next day I could scarcely believe my own eyes when I found the bottom of the jars swarming with little Limuli.

## The Ovary.

1. *Position and general appearance.* — The ovaries of Limulus (Pl. XIII, Fig. 2, *ov.*) communicate with the exterior by means of two horizontal slits, situated on the posterio-dorsal side of the operculum, on each side of the median line, and approximated to within one-fourth of an inch of each other. Each external meatus (*g.o.*) is guarded by an upper and lower thickened lip, that is somewhat prominent externally ; and the orifice is further closed by transverse ridges within.

From these two openings, the two terminal oviducts, lying beneath the outer integument of the posterio-dorsal surface of the operculum (*op.*), traverse the proximal part of the operculum, and, proceeding forward, upward and outward, enter the cephalothorax. Here each duct soon divides into two large branches, one of which takes a peripheral direction, while the other takes a course toward the central axis of the body, where it anastomoses with the corresponding branch of the opposite side, in the median line directly above the alimentary canal (*al.c.*). The point of divergence of the central and peripheral branch of each duct is entirely obliterated, giving the two branches the appearance of one continuous tube, in the middle of the ventral side of which is inserted the slightly larger terminal duct. Between the point of insertion of the terminal oviduct (*ov.d.*)

and the point of anastomosis of the right and left branch, each of these branches gives off a large secondary branch (*ov.t.*), which passes backward on the right and left side of the alimentary tract to the anus (*an.*). These are not simple tubes, however, as has been affirmed by Owen ; but rather a network of anastomosing tubes which surrounds the alimentary tract, nearly concealing it, except immediately over that part of the operculum where the external openings are situated (*g.o.*). The lateral tubes of this system when filled with eggs are larger than the tertiary branches above and below.

After anastomosing in the median line the two secondary branches previously described again separate, and, retaining approximately their original size, pass forward on each side of the alimentary tract (*al.c.*), along the adductor muscles (*m.*), in the meantime sending smaller anastomosing branches over the alimentary tract, and large branches between the muscles. Finally they unite again in the anterior part of the cephalothorax immediately over the oesophagus.

The right and left peripheral branches of the divided oviduct, retaining for some distance the original dimensions, proceed outward, slightly backward and downward, giving off, close to the adductor muscles, a large branch. This passes along the muscles to the right and left of these till they become united in front of the adductor muscles to the tubes previously described. They again give off branches passing between the adductor muscles toward the central axis of the body; and these unite with the corresponding tertiary branches proceeding from those running parallel with the intestine previously described.

This system of large tubes lying over the alimentary canal and surrounding the adductor muscles has been described by Owen ('73) as the ovary of Limulus.

In the further course of the peripheral branch of each of the secondary branches numerous tertiary branches are given off. The whole finally resolves itself into a number of small tubes that anastomose with their neighbors and with tertiary branches given off from the large system of tubes surrounding the adductor muscles. Thus the whole ovary becomes a net-

work of tubes covering the whole ventral and dorsal surface of the animal, and, uniting at the extreme borders of the cephalothorax, enclose the massive liver and the other internal organs.

The ovary, whose double nature is evident only from the two terminal oviducts, has a bilateral symmetry; but this is incomplete, because of the somewhat irregular anastomoses over the median line.

The secondary branches, surrounding the adductor muscles and running parallel with the alimentary tract, are comparatively large. Owing to their relatively thick walls, they maintain more or less uniform dimensions, even when filled with eggs. These being branches of the oviduct, they serve, like the latter, as reservoirs and channels of transmission for the vast number of eggs that are discharged into them by the numerous tertiary branches. This network of tertiary branches (*ov.*), covering the entire animal outside of the adductor muscles, is the real egg-producing portion of the ovary. A portion of one of these tubes is represented in Pl. XIII, Fig. 16, drawn with a camera from living material, taken from a young animal thirteen inches long, including the tail.

In the adult animal these tubes are usually filled with eggs. Owing to the feeble resistance offered by their thin walls, the eggs in them are not evenly distributed along the lumen, but are often massed together into large masses, causing irregular swellings that obliterate the meshes between the tubes. This gives the ovary a very irregular appearance, as if it were nothing else than a huge mass of eggs covered by a thin membrane. Over and between these large masses of eggs the various stages of new generations of eggs can be seen.

After the discharge of the eggs this chaotic appearance of the ovary, for the most part, disappears, and most of the irregular sac-like swellings resume the normal dimensions of the ovarian tubes. These now become conspicuous, not only from the shining aspect of their walls, but from the fringes of the various generations of new eggs that dot their surface. In the adult animals, except such as have recently moulted, the tubes (*ov.t.*) are never completely collapsed, but contain a larger or smaller number of eggs. These, when not very numerous, are

arranged in rows along the lumen, like so many intersecting bead strings. The arrangement and appearance of the completely empty tubes can best be observed in a half-grown animal, where no eggs have yet been discharged into the tubes.

This network of tubes is suspended between the carapace and the liver in a subcutaneous alveolar tissue, to be described more minutely later. In the turgid condition of the ovary this tissue becomes greatly flattened. The entire space between the liver and carapace is occupied by the enormous mass of eggs, the peritoneal tissue being reduced to a thin film. This does not entirely obscure the eggs when the carapace (*ca.*) is removed.

2. *Muscle coats.* — The terminal portion of the oviduct is characterized by the firmness of its wall. This is due to a highly developed muscle coat (Pl. XIII, Fig. 15, *m.c.*). This coat consists of an outer tunic, underneath which is a thick coat of muscle fibers woven together, apparently without much order, into larger bundles that intersect and cross each other, leaving vacant meshes between. These meshes, however, are small as compared with the muscle bundles themselves. In cross section the cut end of these muscle fibers and bundles show various outlines, corresponding to the various degrees of obliquity in which they have been cut; but no trace of a differentiation into a longitudinal and circular zone is to be recognized. Sections of the larger branches of this duct, however, show a distinct, rather thin inner, circular muscle coat, outside of which is a thicker zone of intersecting and dividing muscle fibers, with connective tissue, blood lacunae, blood vessels, and capillaries. The outermost coat consists, for the most part, of longitudinal muscle fibers, the whole forming a muscular wall considerably less in thickness than that of the terminal duct.

This same muscle coat is continued over the ovarian tubes proper, but in a very loose and attenuated form (Pl. XIII, Fig. 15, *m.c.*). In both transverse and longitudinal sections of the ovarian tubes the same features present themselves. This is true chiefly in the mature ovary where follicles have already been formed. The distinctive feature is that the fibers,

both in longitudinal and in transverse section of the tube, are cut transversely or obliquely, longitudinal sections of the fibers rarely appearing. The cause of this becomes plain when the tube is slit open, spread out on a slide, and stained *in toto*. Those large sac-like swellings previously mentioned have been selected for this purpose. Killed in picro-sulphuric, and hardened in alcohol, the wall of the greatly expanded tube remains sufficiently tough to allow its removal from the mass of contained eggs; and its elasticity is sufficiently destroyed to prevent its return to its normal contracted state after removal of the eggs. Such a preparation, stained with the Biondi-Ehrlich mixture, shows the following arrangement of the fibers. Very symmetrically arranged oval areas are observed where no fibers are present. Around these areas the fibers run parallel to one another, constituting a sort of striated rim or border around the area. Between these areas with the encircling muscle fibers the fibers cross and intercross, some becoming continuous with the encircling fibers of one area, others passing around another area, and so on. The beginning and the end of these fibers could not be made out. They seem to branch freely and have nuclei imbedded in their substance.

The oval areas are due to the characteristic arrangement of the muscle fibers; and this arrangement is the expression of the regularly arranged follicles in the adult ovary of Limulus. The oval areas themselves are the follicles which have become obliterated through the stretching of the walls of the ovarian tubes. That the muscle fibers retain this characteristic arrangement in the wall of the tube, when greatly extended, is perhaps sufficient evidence that these are permanent features of the muscle coat, and not, as might be supposed, transient features due to displacement by developing eggs and likely to occur at any point where an egg might chance to develop. In the adult ovary it is this arrangement of the muscle fibers which determines the position and makes possible the characteristic follicles of Limulus. Something more concerning the origin of this arrangement and the part which the growing egg may have in its production is to be considered in connection with the development of the ovary.

3. *The peritoneal coat.* — As will appear more clearly in the account of the developing ovary, the muscle coat of the ovarian tube is surrounded by a loose coat or mantle, belonging to the honeycombed peritoneal tissue. This has been mistaken for the tunica propria by Kingsley ('92). It serves to suspend the ovary between the carapace and the liver. This tissue is seen to be laminated, and consists of greatly flattened cells, joined edge to edge like the peritoneal linings of lymph spaces generally. The meshes of this laminated tissue appear to serve as lymph sinuses, and are filled with granules and corpuscles of various kinds. It is between two such lymph spaces that the ovarian tube lies. The tube is organically connected with this tissue along one of its sides, so as to hang suspended in a loose tube, the walls of which constitute the walls of neighboring lymph spaces. This peritoneal mantle or peritoneal coat of the ovarian tube is comparatively loose; and it is the looseness of this coat which permits the eggs, as they develop, to push out through the follicular fenestrae of the muscle coat, and to occupy the space between the ovarian tube and encircling peritoneal coat. Because of its looseness, also, the ovarian tube is enabled to greatly enlarge when the eggs are discharged into the tube. In dissecting out the ovarian tubes, the connection of the tube with this peritoneal mantle is usually severed and the mantle does not appear in connection with the ovarian tube. In one sense, it belongs rather to the system of lymph sinuses than to the ovarian tube; but its relation to the ovarian tube is such that it serves a double purpose. The nuclei in the cells of this coat are conspicuous. The corpuscles and granules, so conspicuous in the lymph spaces, are not found within the periovarian cavity bounded by the peritoneal coat.

4. *The lining epithelium.* — In the adult ovary, in its empty and contracted state, the tunica propria becomes folded between the follicles, owing to the tonicity of the muscle coat.

The folding is especially prominent around the borders of the follicles, where rachis-like projections appear to extend into the lumen of the follicle.

This folding causes a considerable lateral pressure on the epithelial cells, which thus become greatly elongated and compressed.

On the expansion of the ovarian tube, this lateral pressure is relieved and the cells assume a more spherical form.

The size of the epithelial cells is subject to considerable variation, and the thickness of the epithelium also varies within considerable limits. This is due, in part at least, to the varying lateral pressure on the cells, and on the amount of folding of the tunica propria; for, where the tube is distended, the epithelium may become flattened into what appears to be a mere thin protoplasmic layer with scattered nuclei. In other portions of the same tube, where the folding and lateral pressure still exists, the epithelium appears to have considerable thickness. In all cases, however, it consists of a single layer of cells.

This epithelium can always be distinguished from all other tissues by its glassy transparency, except at certain stages, when the cell protoplasm becomes filled with secretion granules. The minute structure of the protoplasm assumes different appearances, as the cells are seen to be compressed or expanded. The distinctness of cell boundaries also varies with the amount of lateral pressure. Where the tube is expanded, and the cells are more than usually flattened, cell boundaries are difficult to make out. The cytoreticulum of one cell area seems to be continuous with the cytoreticulum of neighboring cell areas, the nuclei in each affording landmarks. Careful examination of this protoplasm seems to indicate that the various nuclei are connected by means of this system of cytoplasmic fibrils. These fibrils can be seen to be massed into strands or bundles. The bundles, however, can be analyzed into finer fibrils connecting larger microsomes. An increase of the magnifying power shows that the meshes of these fibrils are again traversed by still finer fibrils. At each node there is always a stainable, spherical enlargement, which usually diminishes with the fibril under consideration. The whole has thus the appearance of a network within a network, through a considerable series of gradations in size, both of the fibrils and their nodal enlargements. There appears to be no limit, except that which the microscope imposes, to this continued decrease in the size of the fibrils, the size of the stainable nodes, and the size of the enclosed meshes.

The terms fiber and fibrils have been here used as descriptive terms for appearances as they present themselves under the microscope; not in any way to designate the real character of the appearance.

In the relaxed state of the ovarian tube, and in the folded condition of the basement membrane, this appearance can no longer be discerned. With the increase of lateral pressure the epithelium becomes thicker, and faint indications of distinct cell outlines present themselves. The fibrous network described above is either very obscure or else not visible at all. In the latter case the cell contents appear homogeneous, with granules scattered throughout the protoplasm, and a distinct apparently vesicular nucleus situated nearer the base of the cell. But this is not a permanent condition. Cell outlines become more and more distinct. The granules become larger, clearly defined, uniform in size, and stain with some difficulty, except in such powerful stains as acid fuchsin. They often have a more or less bead-like arrangement. As the granules increase, however, they form large aggregations, occupying nearly the entire cell, and often obscuring the nucleus. At this stage the cell can often be seen to be distinctly striated. The fibers to which this striation is due run parallel with each other, and with the long axis of the cell, perpendicular to the basement membrane. The cells increase greatly in size, and, varying with the lateral pressure, their long axis may be many times that of the short or transverse axis. The form and apparent size of the cell vary with the position which it occupies, and the consequent variation in pressure. Its short or transverse axis may be equal throughout, or the cell may be greatly narrowed at the base and expanded at the free end.

At this stage the free end of the cells has a regular, clean-cut outline, and the fibers of the cell body appear to extend to the very surface of the cell. This, however, is not a permanent condition of the cell; for the free border gradually becomes ragged; the fibers appear to be continued into the lumen of the tube, in which a fibrous, deeply staining substance appears to have been secreted. With this substance the cell becomes apparently more and more continuous. The free border of the

epithelium, previously distinctly and uniformly outlined, now becomes obscure and ill-defined; the fibers of the cell body become more distinct and fewer in number; cell boundaries seem to become very indistinct, and large, open, non-stainable spaces appear to occupy the larger portion of the former cell. The remaining fibers appear packed along one side of the former cell, and in this remnant of protoplasm the somewhat obscured nucleus lies imbedded. The whole epithelium in section now has a very irregular, ragged outline.

Owing to these changes in the epithelial cells, the same method of preservation has different effects, not only on the cytoplasm of the cell body, but also on the nuclei. In well-preserved conditions of the cell, previous to the considerable accumulation of granules, the chromatin consists of deeply stainable spheres arranged in a circle around the periphery of the spherical, vesicular nucleus, forming in haematoxylin stains a dark beaded ring around a clear central area. At times, but not always, a dark body more minute than the chromatin granules can be seen to occupy the center of this clear central area.

After the accumulation of the granules in the cytoplasm, however, this expanded spherical condition of the nucleus and the regular peripheral arrangement of the chromatin is not to be observed. The nucleus seems to collapse, become oval, and the distribution of the chromatin becomes irregular. The chromatin bodies themselves, losing their uniformity in size and their regular spherical form, appear as if broken up. They may also become aggregated into a more or less homogeneous mass. This latter condition of the nuclei is often to be observed in those cells of the egg stalk which show evidence of gradual disintegration. Since the other forms of the nucleus are found in their immediate vicinity, this peculiarity cannot be attributed to the reagents used. Certain methods of killing, however, as, for example, Ehrlich's bichromate, when allowed to act for several days, give to the chromatin of all the nuclei an appearance resembling this latter form.

5. *Formation of follicles.* — The pressure caused by the tonicity of the muscle fibers is greatest between the follicular

fenestrae. The epithelium over the area of the fenestrae, being relieved of this pressure, protrudes through these as evaginated pouches (Pl. XIII, Fig. 15).

The cells lining these follicles are spherical, as compared with the greatly elongated, compressed cells between the follicles. The cell outlines are not so distinct as in the elongated cells, and the epithelial lining of the follicle often appears more like a layer of protoplasm containing nuclei than like a well-marked epithelium of columnar cells.

In very many cases these follicles are filled with a secretion resembling yolk. This secretion is seen to arise in the cells of the epithelium often in the form of granules, and frequently in the form of a non-stainable mucus-like substance, which is accumulated in large masses, occupying nearly the whole cell. The secretion which is poured into the lumen of the follicle may be discharged through the communication of the follicle with the lumen of the ovarian tube into the latter, the follicle in such a case serving the purpose of an ordinary gland. There is no reason for supposing that these lining cells of the follicle differ in any essential respect from those lining the ovarian tube proper, since their difference in form can be accounted for by their freedom from pressure. The epithelium lining the follicles and that lining the tube proper are, in fact, a continuous layer of similar secreting cells, the peculiar arrangement which they have being due solely to the fenestrated nature of the muscle coat.

It frequently happens that, instead of being an open space as it is often found to be, or else a space filled with secretion, the lumen of the follicle is filled with large masses of protoplasm in the form of large, ill-defined, irregular cells (Pl. XIV, Fig. 23). These cells, occupying the entire follicle, are also seen to be secreting, inasmuch as they become filled with yolk-like granules (*s.g.*), often to such an extent as to entirely obliterate cell boundaries. In such cases the granular secretion appears to arise and accumulate around the nucleus of each cell to such an extent as to obscure the nucleus, and often to render its detection difficult. The chromatin granules of the nuclei are seen between the secretion granules, often apparently imbedded

in their substance. The secretion takes a dark coloration in osmic acid, and, like the secretion of the ordinary epithelial cells of the ovarian tube, stains with difficulty, except with such powerful stains as acid fuchsin. Occasionally such a follicle is seen to have been transformed into a mass of non-stainable, homogeneous substance, showing irregular dividing lines, perhaps the original cell boundaries. These lines, probably the last remnants of the protoplasm of the cells originally present in the follicle, stain in haematoxylin. At their intersections are observed deeper staining patches that might suggest the presence of nuclei.

The large accumulation of secretion here described occurs only in those follicles where an egg is absent.

*Development of the ovary.* — In a young animal of one inch or less there are indications of a subcutaneous alveolar tissue between the liver and the carapace. At this stage numerous deeply staining nuclei can be observed lying between the carapace and the forming liver. The body of these cells can be seen to be flattened, and the various cells bear a certain fixed relation to one another. The meshes between the cells increase, owing perhaps to accumulation of liquids. It then appears that the cells, originally closely packed, now form the thin walls of vesicles or cavities. The nuclei are still prominent. Each of these vesicles has a wall of its own, composed of flattened cells set edge to edge, very much as in the case of lymph spaces in general. As these spaces increase in size the cells of their walls become flatter and the nucleus less distinct. Where two of these spaces are contiguous the wall appears double, as if the two walls had become closely applied.

In sections of the young animal at this stage, and earlier, there can sometimes be seen between these contiguous walls isolated cells with a distinct, deeply staining nucleus, and a well-defined cell body of protoplasm, having that characteristic clearness which is peculiar to the germ cells during the period of division, previous to the period of growth. The position of these cells corresponds to the position of the ovarian tubes, when they can first be definitely recognized as such.

In a series of transverse sections the cells are not to be

seen in all sections, but, in favorable cases of tangential-longitudinal sections, they appear to be arranged in a chain between the lymph spaces. Sometimes it appears as if every other cell belonged to opposite walls of the contiguous lymph spaces; and, being separated considerably, they alternate in such a way as to appear to be a single chain of cells. It seems highly probable that these cells give rise to the future ovarian tubes.

I have not been able to trace the origin of the tubes, but it seems to be somewhat as follows: by division these cells give rise to chains of cells; which chains, consisting of perhaps four cells in cross section (possibly enclosing a cavity or lumen), take the direction of the lymph sinuses between whose walls they lie. But the walls of these lymph spaces intersect in various ways and at nearly all angles. The chains or tubes, therefore, also intersect in the same way, and thus a network is established. The cells continue to proliferate, and the number of cells in cross section increases. Such minute ovarian tubes, from four to six cells in cross section, can be seen in young animals from four to five inches.

With the increase in cross section the peritoneal walls between which the tubes lie become more and more separated; and these walls, originally closely applied as the boundaries of lymph spaces, become the peritoneal mantle, or loose peritoneal coat of the ovarian tubes of the adult animal.

The cells of the rods early show an arrangement as far from the common center of the long axis of the tube as space will permit; and thus a lumen early makes its appearance. Cells can be seen to continue dividing by karyokinesis, and, becoming pushed into the lumen, temporarily obliterate it. In animals from five to seven inches the lumen of the tube is already well established. The cross section of the tubes does not increase equally in all tubes, and their development takes place more rapidly on the dorsal than on the ventral side.

When the cells are sufficiently numerous in cross section to constitute a tube, they are seen to be surrounded by a second membrane, the tunica propria. At this early stage the tunica propria is closely applied to the original peritoneal coat, although they are easily seen to be entirely distinct, the peri-

toneal coat being conspicuous by the distinct nuclei of its cells, while in the second coat, or tunica propria, no nuclei can be seen (Pl. XIV, Figs. 33–41, *t.p.*).

Comparing the tube with its tunica propria, with the walls of the enclosing lymph spaces, it is seen to differ from these in this, that while in the latter the body of the cells is greatly flattened (Pl. XIV, Figs. 37–41, *p.c.*), so that the nucleus finally seems imbedded in the outer lamella, in the former the body of the cell remains conspicuous, and the nuclei are accordingly considerably removed from the common investing membrane, the tunica propria. In the peritoneal cells the intercellular or cementing substance is formed between the cells, which thus become adherent at their circumference; while in the germ cells this intercellular substance appears at the outer pole of the cell, which thus retains more of its spherical form.

In some of the tubes of an embryo seven inches long, traces of muscle cells can be seen between these two coats; and this is, of course, the future muscle coat of the ovarian tube. As the animal increases in size the muscle coat becomes more and more pronounced.

Even in an embryo five inches long it can be seen that some of the germ cells have passed the period of multiplication, and have entered on the period of growth (Pl. XIV, Fig. 38). As they grow they push their way out, causing a separation of the peritoneal membrane and the tunica propria on either side (Pl. XIV, Figs. 40, 41, *p.c.*, *t.p.*), and this circumstance seems to determine the position of the follicular fenestrae of the muscle coat (Pl. XIII, Fig. 15, *m.c.*, *t.p.*). It might be asked whether the increased pressure thus produced between the egg and the peritoneal membrane, and the resulting diminution of pressure on the other side of the egg, has anything to do with the peculiar arrangement of the muscle fibers. It will be seen later that these oöcytes make their appearance at regular intervals as the animal grows; and that this is intimately connected with the increasing diameter of the ovarian tube. But the further account relating to this will be introduced with the history of the growing egg.

## The Egg.

The germ cells lining the ovarian tubes of the young animal when the lumen has appeared are spherical in form (Pl. XIV, Fig. 38). The cytoplasm of the body is relatively abundant in the resting state, and is comparatively free from granules. The nucleus occupies approximately the center of the cell. It is not relatively large, but it contains considerable chromatin, which stains deeply.

As these cells prepare for division the nucleus becomes greatly enlarged, and the chromatin assumes a distinct and highly characteristic network (Pl. XIV, Fig. 33). Dark, straight fibers are seen to intersect other straight fibers, at various angles; and these, again, can be seen to unite with similar fibers at the periphery of the nucleus. Here dark, bead-like chains of chromatin granules appear to constitute the only boundary of the nucleus. These bead-like bodies are not always evenly distributed, but considerable spaces sometimes appear between them. By adjusting the focus, however, similar granules appear in these spaces, indicating that we have here to do with a network which lies at the boundary of the nucleus. At the points of intersection of the chromatin rods within the nucleus there is always a considerable enlargement. The karyolymph is hyaline, and the chromatin element does not appear to be so abundant as to make the nucleus as conspicuous as it becomes in the next stage (Fig. 35). Now the nucleus, retaining its former size or even slightly increasing, is seen to be filled with a deeply stainable thread which appears to have the form of a coil and which fills the nuclear space. It could not be determined whether this is a single thread or several threads. Appearances seem to show that there are more than one thread; at any rate, several apparently free ends could be seen at all times. Careful examination of this thread shows distinctly that it consists of spherical bodies arranged in single rows closely applied so that in places the rod seems continuous; but the granules are often slightly separated one from the other. This, in all probability, may be taken to be the preliminary phase, spireme, of karyo-

kinesis, which follows in the ordinary way (Pl. XIV, Figs. 36, 37).

Unfortunately, notwithstanding the comparatively large size of the nuclei in the earlier stages of this division process, the spindles are small; and the hyaline nature of the cytoplasm renders it difficult to determine the conditions at the poles of the spindle. The difficulty is further increased by the rather peculiar phenomena of several contiguous cells seeming to divide at the same time. Occasionally, single cells in the various stages can be seen; but, as a rule, a group of from four to eight contiguous cells are in precisely the same phases of division. The spindles seem to lie in all planes; and the closeness of the elements renders it difficult to observe an entire spindle with its two poles. Sections of the equators of the spindles showing the chromosomes are numerous; but the confusion arising from the crowded condition of the elements is such that any attempt at counting the chromosomes belonging to a given spindle would not lead to reliable results. A distinct centrosome imbedded in the accumulation of protoplasm at one pole, however, has occasionally been observed.

The stage with the greatly enlarged nucleus and the characteristic chromatin thread is the most conspicuous one in the process. These have been observed in most of the ovarian tubes of young animals from five to eight inches, and in material preserved in the nitro-picro-sulphuric mixture (see methods), as well as in material preserved in Merkel's fluid. In the latter material the thread is most beautifully preserved; while in the former the archoplasm and cytoplasm of the cell are excellently preserved.

As stated above, these cells usually occur in groups; but single cells, in this phase, can also be observed. The most natural conclusion to be drawn in regard to the cause of these cells occurring together in this way is perhaps that they are the sister cells of an original mother cell; and, being of equal ages, they pass through the same cycle of changes at the same time.

In these groups of cells the large nuclei, occupying nearly the whole cell, seem to lie closely applied to one another (Pl.

XIV, Figs. 33, 35), and on first observing them one might easily receive the impression that they are fusing. The distinctness of the outlines of the nuclei, however, renders it easy to make out almost equally distinct cell boundaries. The protoplasm of each cell is reduced to a thin rim, in which can be seen the few fibers of the cytoreticulum, often not exceeding three or four in number. Owing to the pressure the cell body becomes pentagonal, each side being straight and closely applied to a corresponding surface of a neighboring cell. Yet the cell boundaries are distinct.

These groups of cells can be seen in both transverse and longitudinal sections of the tube. In either case the general appearance of the group is the same. In transverse sections of the tubes only one group can be seen; while in longitudinal sections several groups can be seen at regular intervals along the tube.

In such sections the epithelium on the side opposite the group has only about one-fourth of the thickness of what it has on the side where the groups are situated. This same relation holds also in transverse sections, and it is due to the fact that each group pushes into the lumen of the tube, so as to obliterate it at that point (Pl. XIV, Fig. 35). But in the larger larvae, where the epithelial cells have become considerably elongated in the direction of the lumen, these groups of cells are more frequently enclosed by the protoplasm of neighboring cells as if they had originated beneath them, and pushed their way partly up between them. The group, therefore, does not project freely into the lumen.

In the larva these cells, when they assume the resting stage, acquire the general appearance of the neighboring epithelial cells. They, however, remain grouped together for some time.

After a certain number of divisions (the exact number cannot be made out), one of these cells, usually one lying close to the basement membrane, increases in size more rapidly than the others. Without assuming the characteristic chromatin network, and chromatin coil of the preceding stage, the nucleus enlarges, the chromatin becomes divided into irregular granules that become distributed in an irregular fashion along a

system of netted fibers, but yet more abundant around the periphery of the nucleus. Here some of the chromatin granules become aggregated into a homogeneous mass, which is often closely applied to the periphery of the nucleus, and flattened on that side, but otherwise spherical. This being, no doubt, the first appearance of the nucleolus, the nucleus has, at this early stage, the main features of the germinal vesicle of later stages. The cytoplasm is still rather limited in amount, but a distinct cytoreticulum can be made out. This possesses prominent cytomicrosomes that stain as readily in chromatin stains, especially at the nodes, as the chromatin within the nucleus.

In the cytoplasm, close to the nucleus, the archoplasm with a distinct centrosome can be seen (Pl. XIV, Figs. 42, 43).

8. *Relation of the egg to the ovary.* — On the first appearance of the growing oöcyte, in animals from five to six inches, the ovarian tubes are still round in section (Pl. XIV, Figs. 34, 37, 38), and no diverticula have appeared. As the oöcyte increases in size, however, it sinks more and more below the neighboring epithelial cells, as stated by Kingsley (Pl. XIV, Figs. 34, 38, 39). These, as has been observed, are the sister cells of the same group, and for some time partly enclose the growing oöcyte as temporary follicle cells.

The basement membrane becomes gradually pushed out as the egg grows, until it forms an investing membrane of the egg, which, remaining organically connected with the follicle cells only by a narrow isthmus, appears to lie wholly outside of the tube, between the germinal epithelium and the peritoneal coat (Pl. XIV, Fig. 40). Being still invested with the tunica propria, however, it is still within the ovarian tube, and in fact never leaves it (Pl. XIV, Fig. 19, *t.p.*). As the oöcyte moves outward the sister cells belonging to the group assume more and more the appearance of epithelial cells (Pl. XIV, Fig. 34). New oöcytes within the tube begin a similar career of growth. It thus happens that in an animal seven inches long where some of the tubes have acquired a considerable lumen, two or three stages of these young oöcytes may be observed (Pl. XIV, Fig. 40). Only one diverticulum, in cross section, has yet been

fully formed, and this is, as yet, to be observed only on the tubes of the dorsal side of the animal.

In an animal eight inches long two diverticula in cross section are fully formed.

In an animal thirteen inches long, being about half grown, six diverticula are observed in a cross section of a tube.

Here also can be seen the relation of the ovarian tube to the enclosing peritoneal coat or mantle (Pl. XIV, Fig. 41 ; Pl. XIII, Fig. 15, *p.c.*). The germinal epithelium, with its basement membrane and enclosing muscle coat, is in organic connection with the peritoneal coat only along one of its sides (Fig. 41). Here the various tissue elements become intimately blended, and here, also, blood capillaries and blood vessels are to be seen. At this point the tube increases in size, and it is here that the earliest stages of the forming eggs are to be seen. The epithelial cells are considerably elongated radially. At the base of these cells at this point groups of closely packed, deeply staining nuclei can be seen. Gradually a large nucleus appears surrounded by a definite cell body, which, unlike the cytoplasm of the hyaline epithelial cells, is granular, and stains deeply in the carmine and haematoxylin stains. No evagination of the basement membrane at this point has yet appeared, but the cells lying above the young egg cell seem often to be bounded at their base by a definite membrane, which partly encloses the space in which the young egg lies.

On either side of this point of attachment of the ovarian tube where the first stage of the egg appears, the more advanced stages, in regularly increasing series, are to be seen. Passing around the tube the diverticula increase with the increasing size of the egg, to that point of the tube opposite the point of attachment, where the largest egg in the series is to be seen. There is no connection between the peritoneal coat and the ovarian tube except at the one point of attachment of the latter (Pl. XIV, Fig. 41). The ovarian tube with its diverticula hangs suspended from the inner wall of the enclosing peritoneal coat, along one of its sides. In sections the peritoneal coat is seen only when the ovary is sectioned *in situ*, which is the more convenient in the young forms.

In the adult the relation of the egg to the ovary and of the various parts of the ovary to one another is essentially the same as in the younger forms (Pl. XIII, Fig. 15). As compared with the younger forms the ovarian tubes, in cross section, are greatly enlarged, and the number of diverticula, in cross section, are proportionately increased. But this is not necessarily true of the number of eggs that may appear in cross section, for after the discharge of the first set of eggs into the ovarian tubes a number of empty pouches containing no eggs may be seen (Pl. XIII, Fig. 15). These occupy the same position which the discharged egg previously occupied. Originally they arise, as has been seen, in the young animal by the pushing out of the tunica propria through the fenestrae of the muscle coat as the egg grows. The sister cells of the egg after division of the oögonia become the lining cells of the stalk of the egg. The stalk of the egg, however, exists only during the empty state of the tube, for when the tube becomes stretched by mature eggs that have been discharged into it, the stalk of the egg disappears and its epithelium constitutes the lining epithelium of the ovarian tube.

While it is true that throughout the various stages of growth of the animal the number of eggs in cross section of a tube regularly increases, there appears to be a period beyond which no new eggs are formed. In an animal eighteen inches long eight eggs appear in a cross section of a tube. Several animals, among them a soft-shelled one, equaling in size some of the females that were observed ovipositing at Woods Holl, had discharged no eggs from the follicles, and yet the number of eggs in cross sections had not increased.

Notwithstanding a most careful examination of ovaries from a large number of adult females, collected both at Woods Holl and at New Haven, and showing empty follicles, having evidently reached the period of sexual maturity, I have in no case been able to observe the first stages of the forming egg as it is so easily done in the earlier stages of the growing animal. I infer, therefore, that new eggs are not formed after a certain period, and that this period is either earlier than the period of the first discharge of the oldest egg into the ovarian tube or

else coincides with it. So far as I have been able to determine from an examination of many specimens throughout the growing series, the number of diverticula that appear in a cross section of a tube is equal to, and does not exceed the number of oögonia in the cross section of a tube previous to the formation of an oöcyte.

Furthermore, in the adult animal having empty follicles, the number of eggs in cross section of a tube decreases in proportion as the empty follicles increase; and the size of the smallest eggs is proportional to the number of empty follicles, and inversely proportional to the number of eggs in cross section.

In higher animals it is known that a period exists when eggs for the first time are discharged. It is also known that a period exists after which reproduction does not take place. It is also known that in many higher animals it is impossible to find the earliest stages of the egg in the adult animal, and it frequently has been assumed, on this account, that not only the origin, but the history of the egg in the adult differs radically from the history of the egg of the same animal in its early stages. Thus Balfour ('78), in Elasmobranchs, describes two methods by which the egg may arise: first, by a fusion of a number of cells, which he thinks is the normal process; and, second, by a gradual transformation of a primitive ovum into a permanent ovum.

To enumerate, briefly, the observations: In the young animal, up to five inches, the germ cells form the lining of the ovarian tube. At this period growing oöcytes make their appearance as diverticula, and continue to be formed up to the period of sexual maturity. After this period no new oöcytes are formed; but those already existing continue to grow as the animal grows, until the period of sexual maturity, when the eggs in the follicles first formed are discharged into the ovarian tube. The first oviposition takes place considerably later, and continues at intervals till all the eggs have been matured, which may cover a period of at least eight years. With an intermission of more than one year between the periods of oviposition, as seems probable from the observations recorded

in the chapter on natural history, this period may of course be greatly extended. It is seen from those observations, also, that after the period of sexual maturity, which may be reckoned from the first discharge of eggs into the ovarian tubes, the phenomena of moulting, if not entirely suspended, become at least less frequent. It may be supposed that from the period of sexual maturity the animal does not increase so rapidly in size. That it does increase in size after the period of sexual maturity seems probable from the fact that females that were observed ovipositing differed considerably in size.

The original germ cells (oögonia), up to the time when the embryo measures five inches, including the tail, multiply by an equal division. At this stage they number about eight in cross section. This marks the end of the *period of multiplication.*

At about the sixth-inch stage of the animal a new period in their history begins — the *period of growth.* This is immediately preceded by a multiplication process differing from the former in that the products of division are dissimilar. The karyokinetic processes by which this takes place have previously been described. The result of this process is the formation of a group of cells, one of which becomes the growing oöcyte, while the others belonging to the same group become temporarily the follicle which ultimately forms the permanent epithelium of the ovarian tube. In this way the original germ cell has acquired a new environment, inasmuch as it is henceforth destined to be removed farther from the lumen of the ovarian tube, and is guarded by its daughter cells, which, as follicle and epithelial cells, serve to nourish and protect it.

This transformation of the original oögonia into the protected, specially nourished, and consequently growing oöcyte does not take place simultaneously in all the original oögonia, but it is first accomplished in that one farthest removed from the point of attachment of the ovarian tube (Pl. XIV, Fig. 41). From now on, this first-formed oöcyte continues to grow as the animal grows, and is the first to arrive at that stage of maturity which marks its discharge into the ovarian tube when the period of sexual maturity of the animal is reached.

At the seven-inch stage of the animal this first oöcyte has formed a complete diverticulum (Pl. XIV, Fig. 40). Longitudinal sections of an ovarian tube, when it passes directly in the plane connecting the point of attachment of the ovarian tube and the diametrically opposite side, show a series of these first oöcytes all practically equal in size.

In transverse sections of the tube, in this stage, it is seen that the immediate neighbor on the right is passing through the same process (Pl. XIV, Fig. 34); and this being formed, two diverticula of the ovarian tube may be seen in an animal about eight inches long. Now a third on the left is forming a follicle in the same way. Thus the forming oöcytes with their follicles and diverticula appear alternately on either side of the one first formed. In the thirteen-inch animal five have formed and a sixth is forming; while in the eighteen-inch stage eight diverticula have been formed, the smallest being close to the point of attachment of the ovarian tube.

As these oöcytes increase in size uniformly from the time of their first formation, the one first formed continues to be the largest, the others on either side of this being smaller and smaller, corresponding to the time of their appearance, as the point of attachment of the tube is approached.

The regular sequence in which the oöcytes make their appearance gives to each a definite amount of space, which relieves it from pressure during growth and preserves its spherical form. It is readily seen, also, that this sequence affords a compensation in the economizing of space in the periovarian cavity; for when the first oöcyte attains to a definite size it is discharged; and thus the amount of space by successive discharges, as each in its turn grows, remains practically the same throughout. A portion of an ovarian tube, taken from the living animal thirteen inches long, and examined under the microscope, presents the appearance of an elongated cluster of grapes (Pl. XIII, Fig. 16). In this way, also, it can be seen that the eggs decrease or increase uniformly in size as the tube is rotated on its longitudinal axis.

Up to this point it can be said that there exists a correlation of growth between the parent organism, the

ovary, and the eggs, after they enter on the period of growth.

It is known from the observations of Lockwood, which I can fully confirm, that the young animal moults more frequently in its earlier than in its later stages. This seems probable from the observations related in the chapter on the natural history, where it was stated that the apparently grown soft-shelled specimens were found, on examination of the ovary, not to have any mature eggs in the ovarian tubes; and that animals of an equal size, having moulted earlier, were in a similar condition. Histological examination of the ovaries of these animals showed that no egg had been discharged from the follicles, this being an easy matter to determine.

It was also stated that animals having mature eggs in the ovarian tubes always had hard shells, which was also true of all those females observed at Woods Holl during the spawning season.

These observations seem to show that the moulting is not a phenomenon in any way connected with the season of the year, but that it is intimately connected with the phenomena of growth.

Now, it having been shown that the young of Limulus moult much more frequently than the adult animals, and that with each moult the young animal increases greatly in size, it is extremely probable that the animal increases in size much more rapidly in the earlier than in the later periods of existence.

From the comparative size of the first-formed diverticulum, and its contained egg in the seven-inch animal and the later stages, the same retardation of growth from earlier to later periods, observed in the animal, seems to hold good also in regard to the growth of the ovary and the eggs contained in it.

This may explain the apparent contradiction in the correlation of growth, which at first sight seems to present itself in the case of those eggs that are still growing after the period of sexual maturity is reached. For while the oöcyte first formed is discharged from the follicle at the first period of sexual maturity, the one which is just formed in an animal eighteen

inches long may not be discharged for many years thereafter, even though the difference between the two in point of time of first appearance may be much less.

It is known that in many higher animals, especially in the human subject, precocious growth is often accompanied with precocious sexual maturity, and that this marks an important epoch in the life of the individual. It is known also that this period is evidence of maturity of the sexual organs. In Limulus it seems extremely probable that the discharge of the first egg into the ovarian tube marks the period after which no new eggs are formed. Those already formed continue to grow at the decreasing rate at which the animal increases in size, after the period of sexual maturity. They are discharged from the follicle when they attain to the size which is normal to them; and, continuing thus to be discharged and no new eggs being formed, the time of sterility finally arrives.

On the discharge of the egg from the follicle into the ovarian tube, it is severed from its organic connection with the parent organism and acquires a new environment. Here the egg increases to double its former size within a very short period of time. As will appear later, this change in environment and in the rate of growth is accompanied by marked internal changes in the constitution of the yolk. The important fact to note here is that with the severance of the egg from its organic connection with the parent organism the correlation in growth no longer exists ; and that the egg, having acquired an individual existence, grows at a rate entirely out of proportion to the rate of growth of the animal.

The egg now is surrounded on all sides by the secretion of the epithelial cells; it no doubt utilizes this secretion as nutriment. In studying the structure of the egg, it appears that the egg membrane is radially striated, and that these radial striae are due to protoplasmic fibers that extend out to the investing tunica propria and are in some way connected with it. When the egg is discharged it becomes separated from the tunica propria. This remains behind as the only wall at that point of the tube, and later becomes lined with a new epithelium, perhaps regenerated from the surrounding epithelial cells. The

cavity in which the egg lay becomes practically obliterated by the stretching of the walls of the ovarian tube to accommodate the eggs within it, and only later bulges out as an empty follicle, after the tension within is relieved on the discharge of the eggs in oviposition.

Concerning the rôle which the radial protoplasmic fibers of the chorion may have in the transfer of nourishing material from without, I have nothing on which to base any positive statements. Neither do I know whether these fibers are retracted within the egg, thus leaving pores after the discharge of the egg from the follicle. It may be supposed, perhaps, that they serve somewhat as delicate pseudopodia in the transfer of nutriment. Among others, Eimer ('72) has ascribed such a function to them in the egg of reptiles.

However that may be, the fact remains that the eggs increase greatly in size and become unfavorable for sectioning, a feature that does not exist up to this time.

## Stages of Growth.

The period of growth extends from the last division of the oögonia to form follicles to a somewhat indefinite period after the egg has entered the ovarian tube and has attained its full size. By regularly recurring internal phenomena this period divides itself into four stages. First, a stage extending from the beginning of growth to the formation of the first layer of the egg membrane. Second, a stage extending from the end of the first to the time when the germinal vesicle begins to move towards the periphery. Third, a stage beginning with the gradual approach of the germinal vesicle to the periphery of the egg and terminating with the discharge of the egg into the ovarian tube. Fourth, a stage extending from the time of entrance into the ovarian tube to the time of oviposition.

Each stage may be first briefly described, after which the history of each part of the egg will be considered separately.

*Stage I.* — The most striking peculiarity of the growing egg at the time when it can first be recognized as such is the deeply stainable granular cytoplasm which, previous to growth, is char-

acterized by a peculiar glassy translucency. The germinal vesicle also, at first a nucleus not differing perceptibly from the neighboring nuclei of the follicle and epithelial cells, increases in size and becomes more conspicuous by the increase of stainable substance. Part of this becomes condensed, or separated off and collected into a nucleolus, which previous to this time could not be observed. At this time, also, the archoplasm, centrosome, or vitelline-body, is more conspicuous in the cytoplasm.

Perhaps the most conspicuous feature of the egg, as a whole, in this early stage is the strong affinity of the cytoplasm and germinal vesicle alike for carmine and haematoxylin stains. This peculiarity becomes gradually lost after the first stage is passed. Unlike the nuclei of the follicle and germinal epithelial cells, as well as the nuclei of other tissue cells of the ovary, the germinal vesicle cannot be made to show the green stain of the Biondi-Ehrlich mixture. The loss of this property appears to take place about the time when the nucleolus makes its appearance. The germinal vesicle and cytoplasm stain deeply in haematoxylin and carmine stains up to the time when the first layer of the egg membrane is formed.

In the cytoplasm, during this stage, there is an area, usually close to the germinal vesicle, which does not show this affinity for carmine and haematoxylin stains, but which, on the other hand, has a peculiar affinity for Lyon's blue, picric acid, eosin, acid fuchsin, and erythrosin. At this stage the germinal vesicle is regularly spherical, and its position is usually slightly, but at times very excentric. The proportion between its size and the amount of cytoplasm is perceptibly greater than it is found to be in later stages.

*Stage II.* — In this stage the amount of cytoplasm, as compared with the size of the germinal vesicle, has increased. The cytoplasm is surrounded by a thin layer of dense substance immediately under the investing membrane. The germinal vesicle, instead of being spherical as before, now shows sac-like diverticula that appear like buds on its surface. The nucleolus has increased proportionately in size, and shows changes that are not to be observed in the previous stage. This stage as

contrasted with the previous stage is marked by the considerable loss, by the cytoplasm, of that affinity for chromatin stains, and by the greater size and clearness of the centrosphere. The yolk granules are more abundant and many of them seem to have increased perceptibly in size.

*Stage III.* — In this stage the germinal vesicle is relatively more excentric in position, and subject to great variations in form and size. Compared with the amount of cytoplasm and yolk it is perceptibly smaller. The nucleolus is often very large relatively, and shows many irregularities in form and structure. Numerous "Nebennucleoli" exist. The chorion has increased greatly in thickness by the addition of new layers. The cytoplasm is conspicuously marked by a polar differentiation, one pole being rich in yolk granules and the opposite pole comparatively free from these granules.

At the end of this period the germinal vesicle lies close to the periphery, partly surrounded by a spongy, hyaline protoplasm that does not stain readily. The egg having attained about half of its normal size, but as yet showing no true yolk spheres, is at the end of this period discharged into the ovarian tube. The manner in which this appears to be accomplished has been described above. The egg has now entered on its fourth and last stage.

*Stage IV.* — This stage is marked by a modification of the cytoplasm that renders sectioning in paraffine extremely difficult. This appears to be due to marked changes in the yolk granules. These assume regular spherical forms, and increase very rapidly in size. Owing to the rapid increase of the yolk spheres the egg increases proportionately in size, and this increase in size appears to be a very rapid one. In the first periods after its discharge from the follicle the egg can still be sectioned in paraffine, but the yolk bodies can be seen to have become vesicular and regular in outline, though still comparatively small. The yolk bodies, even now, adhere less firmly to the slide, so that passing the slide through different grades of alcohol or even dissolving the paraffine is liable to wash many of them away. This was not the case in the previous stages. All transition stages from these first definite yolk spheres to

the fully grown yolk spheres can be observed, not in the same egg, but in a series of eggs, according to the time which has elapsed since their discharge.

In this stage the nucleolus has disappeared, and the greatly increased yolk spheres often render it difficult to find any trace of the germinal vesicle, except in the first part of the period, when it still can be seen immediately under the egg membrane or comparatively close to it.

*Degenerative processes.* — In the third stage it sometimes happens that the egg, instead of being discharged into the ovarian tube, undergoes degeneration. This has been observed occasionally in material collected both at Woods Holl and at New Haven; but it was most pronounced in the ovaries of those animals which were obtained from the aquaria of the United States Fish Commission at the World's Columbian Exposition in Chicago.

These animals had been kept in confinement for at least six or seven months. It is probable that they had suffered from lack of nourishment, as well as from other disturbing influences incident to a long confinement.

The ovarian tubes of these animals were filled with mature eggs, and oviposition had probably been prevented by their captivity. Many of the larger follicular eggs show the regressive metamorphosis referred to.

The metamorphic process appears to take place in two ways: first, by the gradual absorption of the egg without the invasion of cells; second, by the appearance, within the egg, of innumerable nuclei (Pl. XIV, Fig. 30).

In the latter case the germinal vesicle, so far as observed, is in all cases absent. On their first appearance the nuclei are found at the proximal pole, where, in this stage of the egg, the germinal vesicle is normally found. With the increase of these nuclei they spread throughout the central part of the egg; and, without at first producing any abnormal appearances of the yolk, gradually fill the entire egg (Pl. XIV, Fig. 30). Simultaneously with this, one or several layers of well-defined, polygonal cells surround the egg, between the outer tunic and the egg membrane, in many cases giving the appear-

ance of a true follicle epithelium. At times this layer of cells may not extend to the distal pole; and in still other cases several layers may appear at various points.

These enveloping cells appear to be continuous with the epithelial cells of the stalk, but their boundaries are more sharply defined. The nuclei of these cells resemble the nuclei of the germinative epithelium, but their cytoplasm is always packed with stainable granules resembling yolk granules. The nuclei within the egg present every similarity to the nuclei of these surrounding cells; and, like the latter, in advanced stages of metamorphosis of the egg they are surrounded by deeply staining granular areas of protoplasm, indicating cell outlines. Often, however, the nuclei are seen imbedded in interwoven strands of protoplasm, where no cell boundaries are visible. This may occur in different portions of the same egg. On their first appearance the nuclei are often uniformly distributed throughout the yolk, in which cases the yolk may be normal, or else slightly broken up into comparatively large masses, giving a vague suggestion of cleavage.

In stages farther advanced the nuclei, which at first showed no indication of cell boundaries, become more or less grouped into patches. The yolk granules, previously evenly distributed throughout the egg, evidently disappear in patches at different times, till one pole of the egg may be nearly devoid of yolk granules. It then shows only the strands of protoplasm with scattered granules, and nuclei imbedded in them; while the other pole may still have the normal appearance, with the exception of here and there an isolated nucleus.

In section, except in the earliest phases of metamorphosis, the outlines of these eggs become irregular (Pl. XIV, Fig. 31). The egg membrane becomes indented, folded, and perforated in various ways. The perforations may pass transversely or obliquely, and in these perforations cells resembling the granular cells surrounding the egg are often observed. These perforations often communicate with spaces between the outer tunic and the infolded egg membrane, which spaces may be filled with granular cells resembling those observed in the perforations.

At the proximal pole the egg membrane is often partially or completely destroyed; and a nucleated mass of protoplasm within the egg appears directly continuous with the protoplasm of the cells lining the stalk (Pl. XIV, Fig. 31).

That these bodies in the egg are real nuclei there is no reason to doubt. They differentiate very excellently with the ordinary nuclear stains. Diluted Delafield's haematoxylin, slightly acidulated, makes them prominent; and they show the differential green stain of the Biondi-Ehrlich triple mixture. From material collected at New Haven, where the animals were in their normal habitat, preparations showing these nuclei were obtained by means of the double stain of Lyon's blue and lithium-carmine, the nuclei alone taking the carmine stain.

The final result of this process of absorption, both where nuclei are present and where these are not to be observed, seems to be the removal of the entire substance of the egg. The last traces that are to be observed are those of the egg membrane, which appears to persist for some time after its contents have been absorbed.

The lymph spaces adjoining the ovary containing such eggs are often seen to be crowded with granular cells resembling very much the granular cells surrounding the egg.

Strahl ('92) found that, in the mature follicles of Lacerta agilis, when the animals are kept in confinement and separated from the males, an atrophy takes place in the mature ovarian egg. The first evidence of this is the disappearance of the nucleus; second, the segmentation of the yolk as in cleavage, and finally the entrance of leucocytes. These at first appear aggregated around the point where the nucleus was situated, but later they distribute themselves throughout the egg.

The segmentation of the egg of the domestic fowl in an unfertilized state has frequently been affirmed, among others, by Oellacher ('72). In these, as well as in the unfertilized eggs of bony fishes, according to him, a division of the nucleus and a real cleavage takes place. The same has been described in the egg of the dove by Motta and Mayo.

Born claims to have observed a cleavage of the unfertilized

egg of the frog. He was unable to state whether this was accompanied by a division of the nucleus.

Balbiani ('93) found that the ovarian eggs of spiders also degenerate ; and he figures follicles filled with cells.

I am unable to make any positive statements in regard to the immediate causes of metamorphosis. I believe, however, that the following statement can be made : the cause of the disturbance lies in the egg itself. In the present case there is no true follicle epithelium surrounding the egg. The cells of the stalk, which correspond to the follicle epithelium in other eggs, and which appear to have a similar relation to the egg so far as the function of nutrition is concerned, seem perfectly normal. They often appear to be unusually active and evidently enter the egg at the proximal pole (Pl. XIV, Fig. 31).

The conditions which make this possible, as it seems to me, lie in the egg itself, and not in an abnormal condition of the follicle cells, as has been supposed by Flemming ('95) in the case of other eggs.

## The Germinal Vesicle.

A network can be distinguished quite early in the germinal vesicle, and the stainable substance, losing more and more its definite form, becomes distributed in irregular granules over this network, and also between the meshes, being especially abundant at the nodes. The stainable substance tends to become massed at the periphery, and especially at one point, where the nucleolus early makes its appearance. As the nucleolus increases in size, the remainder of the germinal vesicle loses more and more its power of staining deeply in carmine and haematoxylin, and is no longer capable of being differentiated, as ordinary nuclei are, by means of the green of the Biondi-Ehrlich triple stain.

During the first stage of the egg the germinal vesicle is spherical and occupies a slightly excentric position when viewed in the plane passing through the centrosome and sphere. In a plane at right angles to this, its position is about central (Pl. XIV, Figs. 34, 38–41).

In the next stage the germinal vesicle shows a tendency to become irregular, owing to the appearance on its surface of numerous diverticula or pouches (Pl. XVI, Fig. 105; Pl. XIII, Figs. 1, 6–8; Pl. XIV, Fig. 24). These are often of considerable size. They are, for the most part, spherical and remain connected with the germinal vesicle by means of a narrow neck or isthmus. The network and stainable granules of the germinal vesicle extend into these, and they are frequently observed to contain pale "Nebennucleoli" (Pl. XIII, Figs. 7, 8).

Very frequently there is an accumulation of stainable granules at one point near the periphery of the germinal vesicle, and this is at times so prominent that it might be mistaken for a second "Hauptnucleolus." It, however, lacks the definite form of the "Hauptnucleolus," and consists of irregular bodies of very different sizes that stain deeply. When this is formed in the central part of the germinal vesicle, the strands of the nuclear network appear to radiate from it as a center (Pl. XIII, Fig. 10). Occasionally this is so marked that it assumes the appearance of an aster. In some cases the granules are less pronounced; and it can then be seen to have all the features of a centrosome and sphere — a deeply stainable central body, surrounded by a clear zone, which in turn is again surrounded by an outer ring, from which the larger strands of the nuclear network radiate. A somewhat similar arrangement of the nuclear network around the nucleolus is sometimes seen (Pl. XIII, Figs. 4, 8, 11). It is especially pronounced in material hardened in Flemming's fluid (Pl. XIII, Figs. 4, 8), but the appearances are by no means confined to such material. The chromatin network seems often to have a centralized arrangement, and the center of radiation may coincide with the nucleolus or be independent of it. When it is found near the periphery of the nucleus, the wall of the latter often shows an indentation in the form of an acute re-entrant angle at that point (Pl. XIII, Fig. 15). In such cases, which are of frequent occurrence, the principal strands of the network can be seen to radiate from this point in a fan-shaped manner. It can be seen that this point is connected with fibers proceeding directly from

the centrosome and sphere in the cytoplasm. It often recalls very forcibly the observations of Auerbach ('96), Leydig ('83, '88), and Rabl ('89).

The nuclear network can also be distinctly seen in the living egg, without the use of reagents, by causing the contents of the egg to flow out. In such a preparation the nuclear network is very distinct, and presents all of the principal features seen in well-preserved material. It is clearer and better defined, owing to the comparative absence of granules which in preserved material obscure it. A germinal vesicle, removed in this way, remains surrounded on its exterior by a delicate network of fibers enclosing yolk granules. These seem to be intimately connected with the germinal vesicle, and render it impossible to obtain the latter entirely free from them. One might ask whether the peculiarly close adherence of these fibers is not due to a direct continuation with the nuclear network.

Everything seems to point to the conclusion that this stage of the germinal vesicle is a period of great activity.

The germinal vesicle, containing a "Hauptnucleolus" and many "Nebennucleoli," and having attained its maximum size, now begins to approach the periphery (Pl. XVI, Figs. 104, 114). It varies much in form and size. At times long pseudopodia-like processes extend radially far out into the body of the egg, giving the germinal vesicle the appearance of a very irregular amoeba. There may be one or several pseudopodia, and they may thin out to such an extent that it is difficult to trace them. The body of the germinal vesicle, in such a case, may be reduced to a small central area, in which the often very large nucleolus may be seen (Pl. XIII, Fig. 5). It may also be greatly extended in one direction, so as to become flattened out into the form of a fish or an arrowhead.

In all such cases the hyaline karyolymph appears to be wanting, or nearly so. The chromatin granules lie closely packed, and the peculiarly distorted body thus takes the stain with avidity. The "Hauptnucleolus" is always present. There is often a strong temptation to regard these peculiar forms of the germinal vesicle as shrunken conditions due to reagents. As they occur, however, in the best preserved material, it is not

easy to regard them as artifacts. Careful study of the living egg reveals none of those movements of the germinal vesicle and nucleolus frequently spoken of in other eggs as amoeboid. The stage under consideration seems rather to be a period of suspended activity on the part of the germinal vesicle, and the processes extending out into the cytoplasm appear rather as the expression of pressure to which the germinal vesicle is being exposed owing to the increasing mass of yolk.

This period is followed later by one of renewed activity, in which the germinal vesicle again becomes filled with the usual hyaline karyolymph, and assumes a more definite spherical form (Pl. XIII, Fig. 14). Having reached the periphery of the egg, it is often comparatively large and is surrounded on all sides, except that immediately in contact with the yolk, by a hyaline, finely spongy protoplasm, which is comparatively free from yolk granules (Pl. XIII, Figs. 11, 14). The contents of the germinal vesicle in such cases show, especially around the periphery, a finely spongy protoplasm, in every respect resembling that surrounding it. It is still surrounded by an apparently well-defined membrane, and contains still a large, deeply staining nucleolus. This alone shows the characteristic stain of chromatin.

The ultimate fate of the germinal vesicle appears to be that its membrane disappears, the larger portion of its contents becomes diffused through the spongy protoplasm. This may be seen as a cap, covering perhaps half of the egg (Pl. XIII, Figs. 11, 14). It persists as such for a considerable period, until the yolk spheres, now increasing very rapidly, occupy practically all the space within the egg. They gradually encroach on the protoplasmic cap till it is reduced to a thin protoplasmic layer immediately under the egg membrane (Pl. XIII, Fig. 17).

The nucleolus having disappeared as such, the last remnant of the germinal vesicle can be seen as a deeply stainable, irregular, amoeboid body, lying in the yolk some little distance below the egg membrane (Pl. XIII, Fig. 12). The yolk surrounding this has a distinct radial arrangement, and this radial arrangement can be traced as parallel striae to the periph-

ery of the egg, where they are continuous with a small mass of hyaline protoplasm. In one or two cases, stainable bodies suggesting chromosomes have been seen in the midst of this radial striation.

In one case, a mass of hyaline protoplasm, free from yolk and having the form of a spindle, was observed imbedded in the yolk some little distance below the egg membrane (Pl. XIII, Fig. 17). The latter showed a perforation running radially through it at this point, suggesting a micropyle. As this is the only trace of such a structure that has been observed, it cannot be definitely stated to be a micropyle. The lumen of the perforation was occupied by a number of small yolk granules.

In the case of other eggs, various causes have been assigned for the movement of the germinal vesicle towards the periphery.

The yolk accumulating at one pole continues to gradually increase until that pole in which yolk does not accumulate and with which the germinal vesicle is connected, becomes more and more flattened out (Pl. XIII, Figs. 11, 14; Pl. XVI, Fig. 104), the hyaline spongy protoplasm, of which it is composed, being forced more and more over the surface of the egg as the vegetative pole increases. This process continuing, the germinal vesicle soon comes to lie under the cap previously described. Owing to the growth of the yolk at one pole, the germinal vesicle and its surrounding hyaline protoplasm, originally near the center of the egg (Pl. XIV, Figs. 20, 24), becomes more and more displaced, the internal portion becoming turned out, so to speak. It might perhaps be designated as an evagination, somewhat like the finger of a glove when straightened out after being turned in on itself.

*The nucleolus.* — In the stages of the oögonia preceding the final division, resulting in the formation of a follicle, no trace of a nucleolus can be discerned (Pl. XIV, Figs. 33, 35, 37). It is first seen in the oöcyte, at the time when the latter has commenced to increase in size. As we have seen, the chromatin at this time loses many of its previous characteristics, both with regard to chemical reactions and general appearance. The chromatin bodies, so far as they retain their regular form,

become imbedded in a more or less viscid (or granular), stainable substance. In this substance the nucleolus makes its appearance, usually close to the periphery of the nucleus (Pl. XIV, Fig. 39). At first it is often flattened on the side next to the wall of the nucleus, but elsewhere spherical, though at times irregular in outline. In this early stage it often appears to consist of granules. How much of this granular substance may be due to the reagents cannot be definitely ascertained. It soon becomes homogeneous and spherical, and takes up little by little a more central position (Pl. XIV). At a very early period in its history it can be seen to be differentiated into an outer and an inner zone (Pl. XIV, Figs. 34, 44). The outer zone seems more dense, and at first it seems like a comparatively thick investing coat of the internal central body. Occasionally two nucleoli of essentially similar appearance can be seen in this stage (Pl. XIV, Fig. 26). In both the investing, homogeneous layer can be seen to be thinned off at one point, so that the internal, central spherical body partly protrudes through the homogeneous covering.

One of these nucleoli usually increases more rapidly in size, and later becomes the only one visible. The growing nucleolus becomes the future "Hauptnucleolus," of which there is usually only one, but in some cases two.

As the nucleolus grows, it retains for a considerable time its spherical form; and throughout the first period usually remains more or less homogeneous, with now and then spherical vacuoles in its substance (Pl. XIV, Fig. 44; Pl. XV, Figs. 83, 87; Pl. XVI, Figs. 99, 100). These vacuoles are not always mere cavities or fluid particles; they may contain solid bodies that stain a deep black in Heidenhain's iron-haematoxylin.

In the second stage of the egg the nucleolus, although it often seems homogeneous, and filled with vacuoles of different sizes, is seen to possess, in a great many cases, a dense outer layer enclosing a central, spherical mass (Pl. XV, Fig. 68; Pl. XIII, Figs. 8, 9; Pl. XVI, Figs. 108, 115, 117, 118. The central mass often has an excentric position, so that the outer homogeneous part, in optical section, has the form of a crescent. This can be distinctly seen in the living egg (Pl. XVI,

Figs. 106, 115, 117, 118). It is one of the most pronounced characteristics of the nucleolus at this stage. In the nucleolus of the living egg the outer crescent-shaped zone appears to be studded with spherical bodies imbedded in it (Pl. XVI, Fig. 108). Whether these are mere fluid vacuoles or solid bodies, cannot be made out. The central body often appears like a vacuole, but more frequently it is granular. The granules vary in size, not only in the same nucleolus, but in different nucleoli (Pl. XVI, Figs. 108, 115, 117).

In preserved material this central body is seen to be solid or composed of granules, as was the case in the living egg. The central body may at times be greatly enlarged. The outer crescent-shaped body then appears as a cap at one pole of the central body. The horns of the crescent, in sections, becoming greatly thinned out, extend along the sides of the central body. At other times the central body is not so large, the outer zone being larger in proportion. The central body may then be elongated into a cylinder-like body with rounded ends, the outer end projecting through an opening in the outer zone at the point where this is thinnest (Pl. XVI, Figs. 111, 113). This reminds me strongly of the observations of Aimé Schneider and Balbiani ('83). The inner end of the projecting body may be simply rounded, or it may be somewhat enlarged. The whole body may be spherical in form (Fig. 112).

In these cases the outer zone stains more deeply than the inner body, except in Heidenhain's iron-haematoxylin, in which the central body takes a dark stain.

The body can often be seen to have been extruded (Pl. XVI, Fig. 110; Pl. XV, Fig. 79). In such cases a cavity, which communicates with the exterior by means of a circular opening, exists in the nucleolus. The extruded body can be seen in all stages of extrusion. When this has occurred, it is sometimes seen lying close to the opening (Pl. XVI, Fig. 110; Pl. XV, Fig. 79).

The extruded body assumes a spherical form, and, except in Heidenhain's iron-haematoxylin, loses more and more its power of staining. Finally, it resembles an ordinary yolk sphere of the last stage of the egg.

I see no reason why this may not be regarded as a so-called "Nebennucleolus." The difficulty with which these bodies stain seems to correspond to the condition of bodies described under that name by various authors.

Besides this single body extruded in this way, it can sometimes be seen that the central cavity of the "Hauptnucleolus" is filled with a number of comparatively large spherical bodies that behave toward stains similarly to the one just described (Pl. XIII, Figs. 4, 8). In one case an opening in the thinnest part of the "Hauptnucleolus" was observed; and some of these internal bodies appeared to be on the point of being extruded (Pl. XIII, Fig. 4). One was lying at the opening outside the "Hauptnucleolus," and another just inside; the rest of the internal cavity was occupied by several of these bodies. They were surrounded by a finely granular substance which was strongly contrasted with the outer zone, this being very thin, but staining deeply.

I cannot say that all "Nebennucleoli" originate in this way. Occasionally one may be seen partly imbedded in the outer zone of the "Hauptnucleolus," and this may occur at any point where the outer zone is thickest.

Similar bodies are found distributed throughout the germinal vesicle (Pl. XIII, Fig. 15). In the living egg they appear as shining vesicles, often occupying diverticula of the germinal vesicle. They can also occasionally be observed in the cytoplasm of the living egg (Pl. XIII, Fig. 3; Pl. XVI, Fig. 112). As I have never seen them actually pass out from the germinal vesicle, I cannot say that they do so.

If the living egg is ruptured, and the contents made to flow out, they can be seen still within the germinal vesicle, and also in its neighborhood.

On a closer examination they are seen to be vesicles, consisting of a delicate membrane, within which are a number of granules, apparently suspended in a liquid. This can be made to flow out when the membrane is ruptured.

The "Hauptnucleolus" increases as the egg increases in size, and, in the third stage of the egg, may often reach gigantic proportions (Pl. XIII, Fig. 5; Pl. XVI, Figs. 104, 114). It

is extremely variable. As a rule, it is spherical; but it may be perforated with holes and cavities. The center is often finely granular (Pl. XIII, Fig. 7). These granules may constitute the entire nucleolus, except a thin outer homogeneous membrane (Pl. XIII, Fig. 6).

Then again the center may be occupied by a relatively small, spherical, strongly refractive body, the outer zone being relatively uniform in thickness. Occasionally, this outer zone, surrounding the central body, is seen to be radially striated. The striae appear to be continuous with the network of the germinal vesicle.

Instead of a central body, there may be a central cavity in which nothing stainable appears to exist (Pl. XIII, Fig. 5). More frequently, however, the central cavity is filled with a network resembling the network of the germinal vesicle, excepting that the meshes are finer (Pl. XIII, Fig. 1; Pl. XVI, Fig. 107). As in the latter case, the fibers of the network are more or less covered with stainable granules, and the meshes between these fibers remain unstained. This caving in of the interior, so to speak, appears at times to continue till the nucleolus is nothing but a thin hollow shell (Pl. XIII, Fig. 6). This shell may be so large as to occupy nearly one-half of the germinal vesicle. Such cases, however, are not frequent. The interior of such a nucleolus is occupied by a chromatin network which in every way resembles the chromatin network of the germinal vesicle.

More frequently, in this stage of the egg, the nucleolus may be seen to have preserved a solid constitution even to the time when the germinal vesicle has reached the periphery. In most cases it is comparatively large, and stains more intensely than the rest of the germinal vesicle. Yet it is often completely honeycombed with little vacuoles. It often appears as if these vacuoles enlarge and flow together. The large nucleolus then appears like a system of variously connected, stainable strands of nucleolar substance, in appearance not unlike a coarse sponge.

In rare cases such a large, degenerated nucleolus is accompanied by another very much smaller, which does not show the signs of degeneration so conspicuously.

In eggs that have been discharged from the follicle into the ovarian tube no trace of the nucleolus could be observed. The last phases described seem to be stages of final dissolution, and absorption of the nucleolus. It seems that the discharge of the egg from the follicle marks its end, as the entrance of the egg into the follicle marked its beginning. Its history coincides with that period of growth of the egg in which the latter remains in organic connection with the parent organism. This would seem to associate it with the phenomena of nutrition and growth of the egg.

There are cases also, in this period of growth, in which there is no nucleolus in the germinal vesicle. Such cases occur when the germinal vesicle is surrounded by a zone of deeply staining granules, which resemble chromatin granules in their behavior towards haematoxylin and carmine stains (Pl. XIV, Fig. 29). Whether this is an abnormal condition, I cannot say. The appearances will be discussed more fully in connection with the cytoplasm. We have seen, also, that at the beginning there may be two similar nucleoli (Pl. XIV, Fig. 26), while later one of these has disappeared. In the second stage of the egg two nucleoli are rarely observed. But we have seen that towards the end, when the nucleolus has greatly degenerated, there may be a second smaller one apparently having recently arisen.

In view of these facts, together with its great variability, it is safe to say that it is not a permanent organ.

The appearances described above seem to show that the nucleolus is not simple, but composite. It consists of a framework of linin similar to that of the germinal vesicle, and a more or less homogeneous, semi-solid, stainable mass, which, accumulating at the nodes of the linin network, flows together into a spherical body, enclosing portions of the linin fibers. Within this mass chemical changes appear to take place which ultimately result in a substance resembling the yolk of the mature egg, and which, like it, assume the form of spherical refractive bodies. These when formed are extruded and give rise to "Nebennucleoli." The chemical or other processes within appear to continue; and the nucleolus, losing substance from

within appears to receive additions from without. Thus a comparatively large hollow shell arises (Pl. XIII, Fig. 6). It would appear almost as if the addition from without is in the form of a precipitate, which becomes deposited on the surface of the nucleolus.

Owing to its relation to the linin network, which is often to be observed within it, the nucleolus may be considered as having a fixed position. Its movements within the germinal vesicle must necessarily be regulated by the linin fibers which constitute its framework. The "Nebennucleoli" appear to lie more or less free in the meshes of the network.

The main feature of both the "Hauptnucleoli" and the "Nebennucleoli" can be seen in the living egg (Pl. XVI, Figs. 106, 108, 115–118).

a. *Summary on the nucleolus.* — 1. The nucleolus appears at the time when the egg begins to grow.

2. It arises as an irregular or spherical mass in an amorphous stainable substance, surrounding the chromatin elements at the time when the germinal vesicle assumes its specific characteristics.

3. There is usually only one, but occasionally there are two in this early stage.

4. As soon as it has assumed a definite spherical form, it is differentiated into an outer zone enclosing a central body.

5. At first the entire nucleolus stains as readily as the chromatin.

6. The outer zone retains this power of staining, but the inner body gradually loses it.

7. The central body (endonucleolus or nucleololus) stains very intensely in Heidenhain's iron-haematoxylin. With double staining of the latter stain, combined with eosin, the entire nucleolus can be seen as a red outer zone and a black or blue central sphere.

8. This central body may become elongated, so as to protrude through the outer zone.

9. It is extruded from the nucleolus, which then appears as a hollow sphere with an opening at one pole.

10. Other similar bodies may form within, and these likewise are extruded.

11. These extruded bodies are the so-called "Nebennucleoli."

12. In carmine and Delafield's haematoxylin they stain feebly.

13. In the living egg they appear as shining vesicles, composed of a delicate membrane enclosing a fluid in which granules are suspended.

14. They have the appearance of yolk spheres; but as they arise at a time long before the yolk spheres are formed in the cytoplasm, they are not yolk spheres.

15. Similar bodies are seen in the cytoplasm at this stage, but they are not permanent.

16. The part remaining after this extrusion retains its power of staining in carmine stains, and may be designated the nucleolus.

17. This often has the form of a crescent.

18. The interior of this, in rare cases, contains no stainable substance, and appears as if it might be a fluid vacuole.

19. More frequently the interior is occupied by a linin network like that of the germinal vesicle, and, like it, having stainable granules imbedded in it or attached to it.

20. The crescent-shaped or circular nucleolus appears to lose substance from within, and to receive substance from without.

21. It may thus become a large hollow shell of stainable substance enclosing a reticulum.

22. The entire nucleolus may often appear as a spherical mass of granules enclosed by a homogeneous membrane.

23. These granules may sometimes be scattered, and lie imbedded in a homogeneous mass resembling the outer membrane. The granules, when they become refractive and lose their power of staining, may be mistaken for vacuoles. These, however, can be stained intensely in Heidenhain's iron-haematoxylin, so that a dark body appears to lie in an unstained vacuole.

24. When only one of these granules exists, it may occupy the center of the nucleolus.

25. Occasionally the outer part can be seen to be radially striated, so that when the central body is present the nucleolus has the main features of a centrosome and a sphere.

26. Such a structure is sometimes to be observed in addition to a second homogeneous nucleolus.

27. When the central body exists, there may be a network surrounding it, which in turn is enclosed by the outer layer of the nucleolus.

28. In such cases the nucleolus has all the features of a germinal vesicle, with nucleolus network and nuclear membrane.

29. The nucleolus can be seen as long as the egg remains in the follicle, but not after its discharge.

30. In the later stages it is often very large and stains deeply.

31. It may, however, become honeycombed with large openings.

32. In such a condition it may be accompanied by a very much smaller one, apparently more perfect, and, like it, staining deeply. This distinguishes it from the somewhat numerous "Nebennucleoli" that are spread throughout the germinal vesicle, and in carmine stains have a yellow coloration.

33. The nucleolus may, therefore, consist of three different constituents: (*a*) linin framework; (*b*) substance resembling chromatin; (*c*) substance resembling mature yolk globules.

34. Movements of the nucleolus are regulated by the linin framework which permeates it.

35. The nucleolus disappears as such when the egg is discharged from the follicle, and when, as we shall see, an entirely different process of growth of the egg takes place.

36. The history of the nucleolus coincides with the period of growth of the egg, *i.e.*, while it remains in organic connection with the parent organism.

b. *Literature.* — An extensive literature on the nucleolus exists, from which many similar observations could be cited. We are reminded at once of the observations of Balbiani ('83) and Aimé Schneider ('75).

I cannot accept Rhumbler's ('93) mechanical explanation of the radial feature of the nucleolus, nor his equally mechanical explanation of the endonucleoli.

Balbiani's explanation of an extruded body in eggs of Geophilus, as being a tube, would lose much of its incredible features if the term tube had not been applied to it. The observations themselves are no doubt correct, but his figures are as diagrammatic as his language is colored by a vivid imagination.

Space will not permit an extended consideration of the many problems concerning the nature and function of the nucleolus. Most of them are well known, and the bearings of these observations will be readily perceived. I would refer the reader to the following authors: Cramer ('48), Ludwig ('74), v. Wittich ('49), Leuckart ('53), Pflüger ('63), Will ('86), Rhumbler ('93), Balbiani ('83), Aimé Schneider ('75), Valentin Häcker ('95), Gegenbaur ('61), Stuhlmann ('86), Bumpus ('91), Waldeyer ('88), Leydig ('55), Brandt ('78), Korschelt ('89), La Valette St. George ('66), Gustav Mann ('93), McFarlane ('92), Goette ('75), Balfour ('78), Henking ('82), O. Hertwig ('77, '92), Flemming ('75), Auerbach ('74), R. Zacharias ('87), Scharff ('88), Mertens ('93), Klein ('78), Holl ('93), Jordan ('93), G. R. Wagener ('79), Platner ('86), Wielowiejski ('85).

## Connection of the Egg with the Ovarian Tube.

Before the formation of the egg membrane the cytoplasm of the egg is continuous with the cytoplasm of the epithelium of the ovarian tube. At times the neck of the egg, by which it is joined to the epithelium, is comparatively large, so that the epithelium appears to lie in direct contact with the egg over a considerable area; and its cytoplasm appears continuous with several of the epithelial cells. In most cases the neck of the egg is constricted to a narrow bridge of fibrous protoplasm, proceeding from the epithelial cells and continuing into the egg body as a polar mitosome. This polar mitosome can often be seen to be continuous with a modified layer of protoplasm surrounding the egg immediately under the investing tunic; and it can also be seen to spread out in a fan-shaped manner in that part of the egg adjoining the stalk. In a few cases the parallel fibers of which the mitosome is composed have been seen to

be connected with a body lying close to the germinal vesicle, the nature of which will be considered later. The polar mitosome is perhaps a remnant of the spindle of the last division of the oögonia, comparable to a similar structure observed by Platner ('86) in the sperm cells of Helix, and by Bolles Lee ('95), or to the Zellkoppel of Zimmermann ('91).

At the junction of the egg with the epithelial cells these fibers are firmly bound together by a body which is prominent in the younger eggs especially, and which stains deeply in acid fuchsin and eosin. In Heidenhain's iron-haematoxylin it stains very deeply, somewhat like the peripheral bodies, which are to be considered presently. If haematoxylin be followed with eosin, it appears as a doubly convex, bright-red body. In many cases sections through its center show the form of a ring. In the younger eggs it is large and conspicuous. It resembles very closely the so-called "Zwischenkörper" of Flemming ('91), which by him was homologized with the cell plate in plants. A similar body was observed by van Beneden in the egg of Ascaris, by Hertwig in Spirochona, by Carnoy ('85) in the spermatocytes of Arthropods, and by Henking ('91) in the spermatocytes of insects. This body does not perhaps differ materially from the numerous peripheral bodies which later make their appearance, and which, as we shall see, give rise to the first layer of the egg membrane. As the latter seem to be aggregations of little spherical bodies, which are the first indications of a forming egg membrane, so this polar body appears likewise to be a concentration of such granules.

*The cytoplasm.* — The cytoplasm consists of at least two distinct elements — a living formed element and a non-living amorphous element (Watasé). The former has the form of a reticulum of variously interwoven fibers, which show a centralized arrangement at the center of the egg. This will be discussed more fully in connection with the attraction sphere and centrosome. The living substance appears to have many of the characteristics of a sponge, in the lacunae, vacuoles, and meshes of which the various amorphous elements are lodged.

*The yolk.* — The yolk lies either massed together at different points, or else uniformly distributed throughout the egg.

It occupies the meshes of the cytoreticulum, and appears to be movable from one point to another, according to the condition of the controlling living substance. It is, therefore, subject to changes in mass, giving considerable variation to the appearance of the cytoplasm. It must be pointed out here that the unequal distribution of the yolk, as well as the variable condensation of the living substance in different parts of the egg, which is frequently to be observed, does not alter the spherical form of the egg. It is hardly probable, therefore, that the spherical form is due to surface tension, which implies an equilibrium of similar molecules in all radii.

In the younger eggs the amount of yolk varies considerably. It may be so abundant as to obscure the cytoreticulum, or it may be very limited in amount. It has been shown that the earliest formed eggs grow more rapidly than those formed in the period preceding sexual maturity. In the latter the yolk is sometimes relatively scarce, and the cytoreticulum is very distinct. In such cases the nutriment of the egg is presumably so limited that the surplus food material is used up in the growth of the living substance. At any rate, it is certain that the egg increases in size by the growth of the living substance, and by mechanical expansion due to the accumulation of yolk. The growth of the cytoreticulum predominates in the earlier stages, while the accumulation of yolk is the chief cause of increase in size after the eggs are discharged from the follicle.

It has been maintained that the yolk originates in all cases within the egg, and it appears to be with considerable reluctance that many, even now, admit the origin of yolk in any other way. This reluctance seems to date back to the early controversy regarding the cell nature of the egg. On the one side it was claimed that the yolk spheres represent real cells; on the other, that the yolk originates within the egg.

An external origin of the yolk has frequently been maintained. I need only mention Ayers ('84), confirmed by L. Will ('84), in the egg of Oecanthus niveus.

There are reasons for believing that in Limulus a substance having the essential characteristics of yolk is produced in the epithelial cells; and that this, in the form of granules,

suspended in a fluid, enters the vacuoles and meshes of the living substance of the egg.

At the time when the first layer of the egg membrane is formed it is sometimes seen that part of the yolk is not included within the membrane, but is cut off and remains outside in that portion next to the stalk of the egg (Pl. XIV, Fig. 22, *y.s.*). The yolk lying outside of the egg in such cases is often considerable in amount, and resembles in every particular the yolk inside of the egg. Two explanations of this appearance suggest themselves, which, although appearing different, may be essentially the same. In the first place, it is safe to assume that the first layer of the egg membrane arises at the extreme limits of the formed, living substance of the egg. Now it may be suggested that in such cases as those under consideration the amorphous elements of the egg extend beyond the outer limits of the living protoplasm, and thus become cut off when the membrane arises. Or it may be that the yolk granules from the epithelial cells, being prevented from entering by the membrane, accumulate outside, later perhaps becoming dissolved and serving as food. It is suggestive that at this stage in the history of the egg, when the yolk granules from the outside are no longer capable of entering as solid bodies, the cytoplasm of the egg undergoes that peculiar change from an alkaline to an acid state of reaction.

The yolk spheres appear in their vesicular, clearly defined form only after the egg has been discharged from the follicle. Previous to this event several of the epithelial cells of the egg stalk appear to degenerate and break up into granules that have all the appearances of the yolk granules of the ovarian egg. On the first appearance of the definite yolk bodies they are small. Those first formed increase in size, and thus in somewhat later stages the yolk bodies may show many different sizes. Ultimately, however, they all attain to a considerable size and fill the egg completely. There can be no doubt that these yolk spheres originate within the egg. Their formation appears to be in some way associated with the new mode of nutrition of the egg after its arrival in the ovarian tube. As previously stated, it is here bathed in the secretion of the cells

lining the ovarian tubes. These contain considerable quantities of such secretions.

The disintegration of cells above referred to recalls forcibly the condition in milk glands, as related by Foster ('93), and also the account given by Nissen ('86).

*Polarity of the egg.*—Adopting the terminology of Auerbach ('96), we have also here a "Kernpol" and a "Gegenpol." The archoplasm and centrosome determine the position of the "Gegenpol." Apparently this is the vegetative pole, for in later stages it becomes especially granular.

At the nuclear pole a hyaline area appears usually in the third stage of the egg (Pl. XVI, Fig. 104). This is often irregular, at times crescent shaped, but not sharply defined from the rest of the cytoplasm. It may partly enclose the germinal vesicle, the horns of the crescent gradually merging into the compact area at the vegetative pole. It often presents a striking similarity to the hyaline area figured by Andrews ('91) in the egg of Diopatra, and recalls the polar rings in the eggs of Clepsine and of Allolobophora observed by Professor Whitman ('78) and Miss Foot ('96), respectively. I do not know how far it could be compared to the polar differentiation observed by Mark ('90) in the ovarian egg of Lepidosteus, which would seem more closely related to the observations of Stauffacher ('93) in the ovarian eggs of Cyclas. Possibly these bodies are more closely related to the "Zwischenkörper" described by me in connection with the egg membrane.

For reasons which are discussed in connection with the cytoplasmic zones and yolk-nucleus, I consider this area due to an infiltration of substance derived from the germinal vesicle. Its position is evidently determined by the relative positions of the germinal vesicle and the sphere. It always appears opposite the vegetative pole. A line might be drawn through the vegetative pole, the germinal vesicle, and the nuclear-pole area (Pl. XVI, Fig. 104). This line, however, would not always pass through the point of attachment of the egg where the "Zwischenkörper" is formed. (See Plates.) The above statements hold true of all stages of the egg from the beginning of growth. (See Pl. XIV.)

As the cytoplasm increases at the vegetative pole, the nuclear pole becomes more and more crowded, till it, with the germinal vesicle, spreads out over the surface of the vegetative pole as previously described (Pl. XVI, Fig. 104; Pl. XIII, Figs. 11, 14).

It is to be remembered that no true yolk spheres exist yet, for these appear only after the discharge of the egg into the ovarian tube (Pl. XIII, Figs. 12, 17).

This process, it seems to me, has some of the features of gastrulation by invagination. The animal portion, less laden with food material, finally comes to lie externally to the vegetative portion. The gastrulation, therefore, might be said to take place previous to fertilization, or even to yolk formation, and the cleavage by delamination, described by Kingsley and also suggested by Brooks ('85) and Bruce ('85), might be regarded as only a continuation of these early conditions. The appearance after the discharge of the egg of a large accumulation of yolk spheres obscures these relations. Yet it is difficult to escape the conviction that a relation of some sort exists between these later developmental processes and the conditions that are found to exist even at the beginning of the period of growth of the egg (Pl. XIV, Figs. 42–47). According to both Kingsley and Brooks, the development of the fertilized egg of Limulus is peculiar in that the first evidence of cleavage appears only on the surface. From what I am able to gather from the accounts of these writers, this division into cells is a secondary matter, the whole egg ultimately being converted into an embryo.

The polarity of the ovarian egg of Limulus is not a matter of chance. It is not acquired during the growth of the egg, but it dates from the beginning. (See Pll. XIV–XVI.) The germinal vesicle alone does not constitute this polarity; for, as has been shown, the centrosome with its cytoplasm exists from the very beginning.

In the history of the germinal vesicle, more particularly the chromatin, I find nothing on which to base the assumption that it is the ovigenic element, and that it is this which presides over all the formative processes. I find no evidence that

the chromatin is the basis of the structure that underlies these polar differentiations. As a matter of fact, the chromatin of the germinal vesicle seems to vanish when the metabolic processes, concerned with the elaboration of food, are at an end.

The peculiar arrangement of the chromatin in the spireme stage, and the various phases of karyokinesis (Pl. XIV, Figs. 33–37) seen in the dividing oögonia do not appear to me to be evidence of an organization existing in the chromatin, but rather an orderly arrangement of an inert mass, brought about by a structural basis which is common to the cytoplasm and nucleus alike.

The division of the oögonia is manifestly a division of the structural basis of both the nucleus and the cytoplasm, and the orderly separation of the chromosomes is due to an orderly separation of the spindle fibers. These spindle fibers I can regard as nothing else than the reticulum of the cytoplasm and the reticular basis of the nuclear network combined. The vital manifestations reveal themselves, not in the passive chromosomes, but in the centrosome, and in the network of which it is a part. The entire history of the chromatin offers nothing on which to base the assumption that it is the controlling element.

The uniaxial feature, which is so prominent in the spindle stage of the dividing oögonia (Pl. XIV, Figs. 36–38), continues to exist throughout the history of the ovarian egg, and can be accounted for only by the assumption of a continuity of structure. It is inherent in the living matter of the egg. Dr. Eycleshymer ('95) has reviewed the literature on this subject, and has tested the relation of the polarity in the amphibian egg to cleavage and to the orientation of the embryo.

*Peripheral bodies and yolk-nuclei.* — On the same slide can be seen the wide contrast between the eggs that are still in the first period and those that have entered upon the second period. In haematoxylin the former are dark blue, while the latter are a very light blue. If the slide be dipped into picric acid, previous to mounting in balsam, the former are not affected, while the latter have yielded their former stain for the new. The same result is obtained by means of borax-car-

mine followed with picric acid. With the double stain of erythrosin and cyanin the same difference can be observed, the eggs in the first stage taking the blue, those in the second taking the red stain. Lithium-carmine and Lyon's blue show the same peculiarity; the eggs in the first stage, in this case, taking the red carmine stain, those in the second stage taking the blue.

While this change in the cytoplasm is in progress, there is a period when portions have undergone the change, while other portions remain in the former condition. In such cases it frequently happens that in haematoxylin stains, while most of the cytoplasm takes a light-blue stain, round, dark-blue bodies resembling nuclei are found scattered through the cytoplasm (Pl. XIV, Fig. 27, *y.n.*). These may easily be taken for nuclei. They, however, disappear as soon as the critical line dividing the first and second period is reached.

I call these yolk-nuclei.

Another class of bodies, which I shall call peripheral bodies, are scarcely less puzzling when first observed. In many cases during the first period in the history of the egg, deeply stainable bodies resembling nuclei are found along the extreme border of the egg (Pl. XIV, Figs. 25, 28 ; Pl. XV, Figs. 66, 67, 77). They are often regularly arranged at equal distances from each other, and always immediately under the surrounding tunica propria or follicular membrane. They stain deeply in Ehrlich's, Delafield's, and Heidenhain's haematoxylin, as well as in carmine and safranin. They do not, except in some rare cases, have the clear outlines of nuclei, but seem rather diffuse (Pl. XIV, Fig. 28). A comparison of a tangential and transverse section shows them to be round discs with one flat side and one convex side, turned inward (Pl. XV, Figs. 79, 90). They are studded with regularly arranged shining dots (Pl. XIV, Fig. 28). At the end of this period the first layer of the chorion is formed. The nature of these bodies becomes evident when the formation of the chorion is observed (Pl. XIV, Fig. 25).

*The egg membrane.* — During the first stage of the egg, its only covering is that formed by the tunica propria, which, as

has been shown, is probably a product of the epithelial cells, being a secretion of the protoplasm of the basal end of these cells (Pl. XIV, Fig. 19, *t.p.*).

The larger ovarian eggs offer excellent opportunities for the study of this tunica propria. When removed from the egg, and viewed in optical section under the microscope, it is seen to be a homogeneous membrane, without cell boundaries and without nuclei. It is, however, studded with closely-set shining dots, as if perforated with closely-set pin holes (Pl. XIII, Fig. 18, *p.c.*). The nature of these becomes sufficiently evident when the formation of the egg membrane is examined.

There being no true follicle epithelium surrounding the egg, the coverings, which in later stages make their appearance, arise from the egg itself. On account of the considerable development of this covering, I shall follow Packard and Kingsley and call it the chorion, being aware that, according to the nomenclature adopted by Ludwig and van Beneden, we should be obliged to call it the vitelline membrane.

The chorion is a product of the egg. In the living egg it has a semi-solid consistency and offers considerable resistance to pressure. It may be ruptured by inserting a needle and severing it in that way. In so doing, it may be drawn out to a sharp point somewhat like india-rubber; but, unlike rubber, it does not return to its former position. Fresh eggs examined in glycerine or normal salt solution often show the formation of extraovates, without the rupture of the membrane.

Examined in the living state, the chorion is seen to consist of one or several concentric layers according to the size of the egg (Pl. XIII, Figs. 12, 13, 17, 18). Each layer is uniform in thickness; but considerable variation may exist between the different layers. The layers appear to consist of a dense substance, between every two layers of which there is a thinner lamella of lighter, apparently less dense substance. These layers of darker and lighter substance are not clearly separated, but grade into each other. Preserved in most hardening reagents, the chorion becomes hard and brittle, offering in the mature egg considerable resistance to the entrance of paraffine or celloidin. In such preparations the lamellae may also be seen;

but they are now separated by clear-cut lines, the less dense, intermediate, lighter layer having apparently been converted into very narrow crevices. In all cases the outer layer, which is the first to originate, differs from the other layers (Pl. XIII, Fig. 18).

Both in the living egg and in the preserved material studied in sections the chorion is seen to be traversed by radial striations (Pl. XIII, Figs. 12, 17). These are closely set and perfectly parallel. In the different concentric layers the radial striations coincide as if continuous one with the other. The striations extend even into the outer or first layer of the chorion (Pl. XIII, Fig. 18).

The first layer of the chorion arises at the end of the first period, and seems to mark an important epoch in the history of the egg, inasmuch as it is at this time that the cytoplasm loses its affinity for haematoxylin, borax-carmine, and other chromatin stains.

It arises in the form of peripheral bodies, which are scattered at regular intervals over the surface of the egg, immediately under the investing tunic (Pl. XV, Figs. 66, 67, 77, 79, 90). These bodies appear at first as minute dots which increase in size, and stain deeply in chromatin stains. They are often so regularly arranged as to be easily mistaken for nuclei. As they grow, two or three may coalesce, forming a conspicuous body at the periphery of the egg (Pl. XV, Fig. 67). As they increase in size by coalescence, they gradually lose their affinity for chromatin stains, and eventually all blend into the first layer of the chorion which, when formed, does not stain readily (Pl. XIV, Fig. 25, *ch.*).

This first layer of the chorion sometimes appears to arise as a continuous layer instead of in patches, as described above (Pl. XIV, Fig. 20). The first indications of its appearance in such cases are not the peripheral bodies, but a layer of deeply staining dots just under the primary tunic. The granules are not concentrated into larger isolated bodies, but are spread out uniformly.

The history of the origin of the subsequent layers of the chorion is a different one, although the process in itself may

be essentially the same. Immediately beneath the first layer of the chorion a homogeneous layer of protoplasm appears (Pl. XIII, Fig. 13), which does not stain so deeply as the more granular protoplasm which it surrounds. In favorable preparations this layer appears to be composed of fibers which resemble those of the cytoplasm except in the absence of the conspicuous cyto-microsomes. The fibers are at first arranged more or less perpendicularly to the surface of the egg (Pl. XIII, Figs. 13, 18). At this stage they present the appearance of regularly arranged cilia, covering the surface of the egg, and they are imbedded in a transparent substance which solidifies into the inner layer of the chorion.

*The radial striations so conspicuous in the chorion of Limulus eggs are therefore due to protoplasmic fibers.* Originally, at least, the radial striations are not due to radial pores, as is so frequently asserted of other eggs. The process appears to be practically similar to the formation of chiton and other cuticular substances by the fusion of cilia. The shining pores previously mentioned in connection with the primary egg covering and the peripheral bodies (Pl. XIII, Fig. 18; Pl. XIV, Fig. 28) are either transverse sections of these fibers or their points of insertion. As we have seen, the outer primordial covering, — tunica propria, — which is the original basement membrane of the germinal epithelium, arises in essentially the same way as a cuticular hardening of the outer ends of the epithelial cells.

The peripheral bodies, which in their earlier stages resemble nuclei, call to mind the so-called "Binnen" epithelium of Eimer ('72), which figured so prominently in the discussions concerning the cell nature of meroblastic eggs, and may possibly explain the much disputed observations of Clark ('57) in the ovarian egg of the turtle. They may possibly be compared to the bodies observed by O. Schultze ('87) and Goette ('75) in the peripheral layer of amphibian eggs, and I believe they serve to explain the observation of Schütz ('82) in the egg of spiders. So far as I am aware, he is the only student of those eggs who has claimed the existence of a follicular epithelium surrounding them. Bruce ('85) and Brooks ('86) have shown

that the "inner egg membrane" mentioned by Packard ('71) is the protoderm, the "rudely hexagonal cells" of Packard being the casts of the ends of the blastoderm cells.

## Zones and Yolk-Nucleus.

The cytoplasm is often divided into two distinct zones — an outer and an inner zone (Pl. XIV, Figs. 19, 21, 24, 29). These two zones are often separated by a distinct line suggesting the presence of a membrane between them (Pl. XIII, Fig. 16). In other cases the zones are separated by a line of large microsomes (Pl. XIV, Fig. 21). This line may run at a uniform distance from the germinal vesicle (Pl. XIV, Fig. 24; Pl. XIII, Fig. 16), or it may be extended at the proximal pole towards the stalk (Pl. XIII, Fig. 16). Instead of this line of large microsomes, the zones may be separated by parallel fibers resembling those of the polar mitosome. In this line the vitelline-body or sphere may sometimes be observed (Pl. XIV, Fig. 21).

The inner zone at times appears less granular than the outer zone, *i.e.*, the granules appear smaller, making the inner zone less stainable than the outer zone (Pl. XIV, Fig. 24). In other cases this inner zone consists of large, irregular, closely packed granules that stain intensely in haematoxylin and other chromatin stains, the outer zone staining less deeply (Pl. XIV, Figs. 19, 29). This form is of frequent occurrence, and is found in the best preserved material, on the same slide with other eggs showing no trace of it.

In the living egg of a half-grown specimen these two zones can be distinctly recognized (Pl. XIII, Fig. 16). In this case the outer zone is translucent, with the exception of a few scattered granules. The inner zone, very sharply separated from the outer zone, is opaque. The germinal vesicle in this case contains a distinct nucleolus. The inner dark zone may form a regular circle around the germinal vesicle (Pl. XIII, Fig. 16, *b*), or it may be extended on the proximal side towards the stalk of the egg, where it seems to become continuous with the protoplasm of the epithelium of the stalk (Pl. XIII, Fig. 16, *c*).

As these eggs were taken from the ovary of the living animal, and examined at once in the fluids of the ovary, the effects of reagents cannot be considered responsible for these appearances.

An examination of the same eggs in a living condition shows also the vitelline-body, or sphere, and its intimate connection with this peculiar inner zone of the cytoplasm (Pl. XIII, Fig. 16, *a*). Pl. XIV, Fig. 21, shows a similar egg in section. The sphere is seen to bear a close relation to the dividing line between the two zones, on the one hand, and to the polar mitosome, on the other.

Eggs showing these zones were preserved and mounted entire. The main features are excellently preserved.

*General considerations.* — The division of the body of the egg into an outer and an inner zone has frequently been observed in other eggs. Among others by Pflüger ('63), cat; Cohn ('56), rotatorian; O. Schultze ('87), Bambeke ('75, '83), frog; Waldeyer ('70), bird; His ('73), fish; Will ('86), Korschelt ('89), insects; Holl ('90), Leuckart ('53), chick; Ludwig ('74), echinoderms; Lancaster ('75), molluscs; K. Schulin ('81), bat and human; Henneguy ('93), fish; Goette ('75), bombinator; J. V. Carus ('50), spiders; Leuckart ('53), Scharff ('88), Eimer ('72), Ransome ('67), fish.

The zones in Limulus eggs present all the essential characteristics of the figures given by the above observers, and may also be seen to undergo many of the modifications that have been observed in other eggs; for example, by Pflüger.

A so-called free space around the nucleus of ordinary cells has also been described; among others, by Leydig ('88) and Brass ('83). A zone around the nucleus of sperm cells has recently been observed by Auerbach ('96).

In the case of eggs the zones are usually considered as being connected with the phenomena of growth and nutrition; but the manner in which they arise is a disputed question.

Leydig and Auerbach take different views. Leydig interprets the inner zone as a free cavity in the cell, into which the nucleus has crowded by a process of budding from the cytoplasm, to which it remains intimately connected by means of a

narrow bridge or stalk. Auerbach, on the other hand, regards it as the expression of a condensation of the cytoplasm, which takes place previous to the division of the cell, and which ultimately results in the formation of a spherical body, the sphere, or "Nebenkern."

Such an explanation of the zone around the germinal vesicle in the egg of Limulus cannot be offered, inasmuch as it appears during the period of growth of the egg, when it has ceased to divide, and often after it has attained to a considerable size. This appears to be true in the fish egg also, according to the observations of Scharff. He considers the outer zone as corresponding to the "Rindenschicht" of Eimer, which the latter believes to be identical with the "Zonoid schicht" of His.

One objection to applying Auerbach's interpretation to the inner zone of the egg is the sharp line which in certain stages separates the two zones (Pl. XIII, Fig. 16). This feature is peculiarly striking in the living egg of Limulus, and is especially emphasized by Pflüger as observed in the egg of the cat, and appears to have attracted the attention of Schulin in the egg of the bat and in the human ovum, and also of Will in the egg of *Colymbetes fuscus*. It is, of course, difficult to say what effect a condensation might have.

The changes of the inner zone from a hyaline to a granular condition, observed by Pflüger in the egg of the cat, and which is so evident in the egg of Limulus (Pl. XIV, Figs. 19, 24), appear to be evidence of chemical changes taking place in the interfilar amorphous substances immediately surrounding the germinal vesicle. This may, of course, be accompanied with an increased condensation of the reticulum; but it would seem that the latter would be more apparent than real, and due rather to the increase in the amorphous granules. The sharp limitation of the inner zone in certain phases of the contained granules, it seems to me, points to differences in composition of the amorphous or granular matrix in which the cytoreticulum lies (Pl. XIV, Fig. 48).

In endeavoring to account for the existence of this inner zone, there are four important elements which demand attention.

If we reject the explanation of Leydig, and also that of Auerbach, how shall we account for the first feature of this zone, *i.e.*, the hyaline stage? Certain features that I have noticed in connection with the germinal vesicle and nucleolus appear to throw light on this question. It was seen that the nucleolus extrudes bodies in the form of vesicles, consisting of a membrane within which is a fluid containing granules (Pl. XVI, Figs. 110–113). These vesicles, which I have called "Nebennucleoli," were seen to vary in number, and apparently to appear and to disappear. There is reason for believing that these vesicles lying in the meshes of the nuclear reticulum finally dissolve, and add their contents to the nuclear sap, or karyo-lymph. Where they happen to lie near the periphery of the nucleus, the discharge of their contents extends the area of the germinal vesicle in that direction, thus causing pouches of the nuclear wall (Pl. XVI, Fig. 105; Pl. XIII, Figs. 3, 6–8). The nucleus, to all appearances, is not bounded by a solid wall, but by a special arrangement of the cytoreticulum.

It has been seen that the germinal vesicle, having become greatly extended, more or less regular in outline, may in another phase of activity become amoeboid, greatly contracted, and apparently devoid of karyo-lymph (Pl. XIII, Fig. 5). In all such cases a hyaline area is found to exist outside the germinal vesicle, apparently caused by the entrance into the cytoplasm of the karyo-lymph, which now becomes either a hyaline zone around the germinal vesicle, or in later stages appears as the polar area referred to in another place (Pl. XIV, Fig. 24; Pl. XVI, Fig. 104).

It would appear that this hyaline zone may become lost in the cytoplasm, in which it becomes diffused throughout the interfilar spaces. We may now consider the question of the origin of the internal granular zone of the egg.

The definite yolk spheres appear only after the egg is discharged from the follicle into the ovarian tube. It is hardly necessary, therefore, to consider the explanation of an internal zone in the egg of the fish offered by His, as his well-known theory of migrating granular cells would not apply in this case, and, so far as I know, has never been seriously considered in

recent years. The theory of Waldeyer also offered in explanation of two zones in the cytoplasm of the bird's egg, and extended also to the observation of Pflüger in the egg of the cat, namely, the direct apposition to the primitive egg cell of the outer zone, conceived to be derived from the follicle epithelium, does not apply in the present case, inasmuch as no true follicle epithelium can be said to be present. Equally inapplicable is the somewhat vague but decidedly radical theory advanced by Leuckart ('53) in regard to all eggs, and the somewhat similar though more metaphysical theory advanced by Balbiani ('83), according to which, in myriapods, the egg consists of a "partie germinative fondamental" and of a "partie nutritive," each of these parts being constituted "isolement et pour son propre compte." The objection to such an explanation would be that in the egg of Limulus the division of the cytoplasm into an outer and inner more or less granular zone is not a constant, but a periodical feature.

It remains to be considered how far the direct elimination of chromatin from the germinal vesicle into the surrounding cytoplasm can explain the existence of the internal granular zone. Such an explanation has been offered by Will in eggs of amphibians and insects, by Bambeke ('93) and by Calkins ('95) in the egg of Lumbricus. Calkins's observations receive increased interest and importance from the evident acceptance of his results by Professor Wilson ('96) in his new work on the cell, and the evident stress which the latter author places upon it in his theory of synthetic metabolism of the chromatin of the germinal vesicle. In addition to the doubt cast upon Calkins's results by the observations of Miss Foot ('96) on the eggs of a closely allied species, Allolobophora, from which she seems inclined to believe that Calkins's results were obtained from pathological material, the following considerations may be urged against the sufficiency of the theory of elimination of chromatin to explain the internal deeply staining zone: 1. It does not explain the existence of the hyaline zone, which appears to precede or to follow the granular condition. The existence of the hyaline zone proves that the zone is not due to the extruded chromatin granules. 2. The elimination of

chromatin granules from the germinal vesicle cannot explain the extension of the inner granular zone towards the point of attachment of the egg, as it can be seen both in sections and in the living egg of Limulus (Pl. XIII, Fig. 16). 3. Contrary to the results of Calkins, the internal granular zone does not behave like ordinary nuclear chromatin towards the Biondi-Ehrlich triple stain. In the degenerating eggs described in another chapter, the chromatin that is found in considerable quantities in the cytoplasm, and having the form of nuclei, does take the green stain with the Biondi-Ehrlich mixture (Pl. XIV, Figs. 30, 31).

I can find no reason, therefore, for concluding that the deeply staining granules of the inner zone are eliminated chromatin granules.

On the other hand, it has been shown that the epithelial cells of the ovarian tube and of the egg stalk secrete a substance which, under the influence of reagents, becomes granular, and that this substance is seen at times to accumulate at the point of attachment of the egg (Pl. XIV, Fig. 22, *y.s.*). From a consideration of the foregoing objections and the facts presented by such appearances as are represented in Pl. XIII, Fig. 16, and Pl. XIV, Figs. 19, 20, 22, as well as from the figures of Korschelt ('89) in the case of insect eggs, I conclude that this secretion enters the egg and is carried along toward the germinal vesicle, where, acted upon by the hyaline karyolymph derived from the "Nebennucleoli," it becomes converted into a stainable substance which I will call *metaplasm.*

While I have called the extension of the inner zone towards the stalk of the egg a channel, I do not mean to imply by that term that it is a tube in the sense in which Balbiani ('83) used the term in the case of the egg of Geophilus. It appears to be rather the expression of the existence of an interfilar fluid or substance, which, for the time being, does not mix with the surrounding hyaline cytoplasmic matrix, and which is especially favorable for the entrance and chemical modification of the crude food material which serves the egg as nourishment. I would not therefore regard the inner hyaline zone as an open space in the sense of Leydig, nor as

a cytoplasmic condensation in the sense of Auerbach, nor as a funnel-shaped tube in the sense of Balbiani, but rather as an interfilar digestive fluid in the sense of Scharff, without the bodily migration of nucleoli as held by him; the granular phases being due to the entrance of food material, as held by Korschelt in the insect egg, and being the result of a combination of a nucleolar product with the nutritive material.

The result of this combination is the metaplasm which later becomes distributed throughout the body of the egg or else collected around the centrosome and sphere.

It is evident, however, that the internal zone is closely related to the centrosome and sphere; and our next problem will be to consider what this relation may be. A relation of some kind has been pointed out by Balbiani, Bambeke, Oscar Schultze, Henneguy ('93), and Auerbach, and is evident from their figures as well as from Pl. XIII, Fig. 16; Pl. XIV, Figs. 19–22, 24, 48, in the egg of Limulus.

In the case of the sperm cell, Auerbach considers the spherical "Nebenkern" as a further condensation of the inner zone. He, however, can give no reason why such a condensation takes place at one place rather than at another; and if I understand him rightly, he does not insist on a structural relation of the cytoreticulum that might serve as a basis for such a condensation.

In the case of the other observers mentioned above, it would seem that they, in a somewhat similar manner, regard the body — vitelline-body of Balbiani — as a fortuitous aggregation of the granules of the internal zone, the granules being regarded either as extruded chromatin or as disintegrated migrating nucleoli.

Against such a fortuitous aggregation can be urged the observation of Balbiani himself, in the case of Geophilus, where a structural body in the form of a centrosome, sphere and aster, is clearly figured and described, in the midst of amorphous granules supposed to be derived from the germinal vesicle. Balbiani does not prove that this sphere and aster originate from the amorphous granules. Furthermore, Mertens ('93) has conclusively shown that a centrosome and sphere

exist, apparently independently of the granules derived from migrating nucleoli.

In the egg of Limulus I have conclusive evidence of the existence, both in earlier and in later stages, of a centrosome and sphere in the midst of the granules of the inner zones (Pl. XIV, Figs. 21, 47, 48); and as will appear from the following considerations of that body, I believe that this is the structural basis around which the metaplasmic granules of the inner zone collect and give rise to the conspicuous body known as the vitelline-body of Balbiani. In that way I would account for the appearances in the living egg represented in Pl. XIII, Fig. 16, *a*.

### The Centrosome and Sphere (Vitelline-Body).

As soon as the growing egg can be distinguished as such, there may be seen in the cytoplasm, in the immediate neighborhood of the germinal vesicle, a body which differs from all other parts of the egg in its staining reactions (Pl. XIV, Figs. 34, 38, 40). It is brought prominently into view by means of Heidenhain's iron-haematoxylin; by the Biondi-Ehrlich mixture; Weigert's picro-carmine; Delafield's haematoxylin, either alone or followed with picric acid; by means of borax-carmine and picric acid; Ehrlich's haematoxylin and acid fuchsin or eosin; by erythrosin, either alone or followed with cyanin; and finally by means of Lyon's blue and lithium-carmine or safranin.

When first observed, it has the form of a crescent closely applied to the germinal vesicle. In the latter double stain it may be made very conspicuous, being differentiated from all other parts of the egg, both germinal vesicle and cytoplasm. These stain red, while the crescent stains a bright blue. In the widest portion of the crescent is a clear area containing central granules (Fig. 42).

This central area, with its granules, appears to be the essential part of the body, inasmuch as it is this which continues to exist in various forms as the egg continues to grow. The horns of the crescent seem to disappear early (Figs. 42–48).

A high magnifying power shows the body to consist of the following parts: first, a round central body, surrounded at a slight distance by a circle of microsomes (Fig. 45). From this circle radial fibers pass out in all directions to another circle of rather large microsomes (Fig. 47). Outside of this again a dense layer exists, which can be seen to consist either of granules or of closely interwoven fibrils, which radiate out into the cytoplasm, and send larger or smaller strands out along the outer wall of the germinal vesicle. In Weigert's picro-carmine, in which the circles of microsomes, as well as the dense fibrous layer, are very distinct, the central granule is not visible. This, however, can be distinctly seen in the carmine and Lyon's blue (Figs. 42–44). After the disappearance of the horns of the crescent, the body appears essentially as before. In Lyon's blue it is a large blue mass of fibers, with a somewhat darker center, situated close to the germinal vesicle, and nearly equal to it in size (Pl. XIV, Fig. 40). It is flattened or slightly concave next to the germinal vesicle ; and it is surrounded by a clearer zone, which is traversed by radial fibers that pass out into the cytoplasm, where they become lost in the red cytoplasmic network. Stained with haematoxylin, followed with picric acid, the cytoplasm and the contents of the germinal vesicle stain a deep blue; the body, on the other hand, appears yellowish (Pl. XIV, Figs. 45, 46). It is seen to consist of granules somewhat closely packed at the center, but less closely packed at the periphery, where the granules are seen to be connected by the same substance as that of the cytoreticulum, into which it passes by imperceptible gradations (Fig. 46). Eggs of the same size as this, however, may show the typical form described above. The indentation next to the germinal vesicle may, however, be so marked as to cause the central granule to lie close to the germinal vesicle (Fig. 45), but yet distinct from the latter, as is clearly seen by the differential stain of Lyon's blue and carmine. At a somewhat more advanced stage, however, the body may be seen about midway between the germinal vesicle and the periphery of the egg (Fig. 49). When stained in Lyon's blue and safranin, all parts of the egg, except this body, take the red safranin stain. The body, however, appears as a

blue or green sphere of interwoven fibrils, in the center of which may be found one or several red granules arranged more or less in a circle (Fig. 49). By means of this double stain, the body can be seen in all stages up to the time of the formation of the egg membrane (Pl. XV, Fig. 79), when the changes in the cytoplasm render the carmine and safranin ineffectual. But even in this case it can often appear very distinctly on account of its greater affinity for the blue, and its consequent deeper stain. In most cases of this kind it appears as a definite, spherical, compact body, consisting of closely interwoven fibers that may at times show a concentric arrangement about a somewhat modified central body (Pl. XV, Fig. 75). There may be two of these central bodies (Pl. XIV, Fig. 58). They appear somewhat like nuclei, each being surrounded by a compact layer of the outer mass of fibers.

In comparatively small eggs stained with various stains, such as the Biondi-Ehrlich mixture or haematoxylin, either alone or followed with acid fuchsin, the fibers of the cytoreticulum can be seen to converge to a point near the center of the egg (Figs. 50, 51, 54, 55). At the point where the fibers meet, a highly refractive body staining deep red in acid fuchsin can be seen. At times when the cytoreticulum is particularly distinct, the body is comparatively small and regular (Figs. 50, 51, 54). When the cytoplasm is more granular, the body may be comparatively large, apparently composed of refractive granules closely packed, and either regular in outline or serrated by projecting processes comparable to the points of a star, the body being very conspicuous because of its greater affinity for the stain than the rest of the cytoplasm, the reticulum of which can be seen to have a somewhat indistinct radial arrangement with reference to it (Fig. 56).

In favorable cases, when the cytoreticulum is especially distinct, the body towards which the fibers of the cytoreticulum converge appears as a ring of dense fibers, the reticular nature of which can, however, be distinctly seen (Pl. XIV, Fig. 32). Within this ring is a delicate network of fibers with distinct microsomes at the nodes, and from the ring surrounded by the cytoreticulum, comparatively straight isolated fibers radiate into

the cytoreticulum, where they can be traced for a considerable distance.

In cases where a distinct refractive body exists at the point of convergence of the cytoplasmic fibrils, this point often lies near the germinal vesicle; and being about in the center of the egg, the latter occupies a somewhat excentric position (Pl. XIV, Fig. 51). The fibers converging at this point, or, as we may say, radiating from this point, are independent of one another over a considerable area surrounding the point of convergence; but they ultimately become continuous with the more reticulated portions of the cytoplasm. An area thus exists near the germinal vesicle, where the fibers radiate; and being less dense, this area, at times at least, appears lighter (Pl. XIV, Figs. 50, 51, 54, 55). The area is usually round, about equal to the germinal vesicle in size, and often flattened or concave at that point where it is in contact with the germinal vesicle (Fig. 50). At times the radial fibers may be very distinct and numerous (Fig. 52). The radial fibers are sometimes definitely limited; but at other times they can be traced to the periphery of the egg, at that point opposite to the germinal vesicle (Fig. 57), the central body being at times inconspicuous (Fig. 52), or at other times apparently consisting of a relatively large granular body into which the radial fibers can be seen to extend at variable distances (Fig. 57). The system of radial fibers is not always so close to the germinal vesicle as described above (Pl. XVI, Figs. 87, 92, 97). The condition of the radial fibers seems to vary. At times they appear as rows of microsomes (Pl. XIV, Fig. 57); while at other times they appear as comparatively homogeneous silken fibers, in which varicosities do not come prominently into view (Pl. XV, Fig. 87). Some of this difference in appearance can no doubt be ascribed in many cases to difference in the staining, but it appears clear that the difference is often due to the condition of the fibers themselves.

In somewhat larger eggs the body appears more complicated. Stained in Weigert's picro-carmine, it consists of a round, dark, homogeneous central body surrounded by a clear zone which again is surrounded by a somewhat lighter zone of granules (Pl.

XV, Fig. 89). This zone of granules is often not so definitely outlined as the central body, but its outer border is often serrated. It is, however, sharply differentiated from the surrounding cytoplasm. From this dark-brown body radial fibers extend into the yellow cytoplasm surrounding it. In Ehrlich's haematoxylin, followed with eosin, the body may show a blue center composed of granules. This is then surrounded by a red zone of concentrically wound fibers more or less interwoven; and from this again red fibers can be seen to radiate throughout the cytoplasm; and in some sections of the latter, where granules are less numerous, the radial fibers can be traced to the very periphery of the egg (Pl. XIV, Fig. 60; Pl. XV, Fig. 67). As the egg grows the body increases in size, and the form described above may thus become relatively large and conspicuous. Stained in erythrosin and cyanin, such a large body may be seen to consist of a large central, spherical mass of granules with a blue tint. This is again surrounded by a comparatively thick zone of bright-red fibers arranged concentrically around the central granules. And around this again can be seen a radial arrangement of the cytomicrosomes (Pl. XVI, Fig. 101).

In Ehrlich's haematoxylin and acid fuchsin the body may appear as a conspicuous deep red, rather small, definite, refractive body in the center of a zone of blue granules (Pl. XV, Figs. 68, 82). The cytoplasm having a less intense red coloration, this form of the body is often a conspicuous and beautiful preparation. From this as a center, also, radial fibers can often be distinctly traced to the periphery of the egg. Many of the red radial fibers can be seen to penetrate the blue zone of granules, and to proceed directly from the red central body (Pl. XV, Fig. 68). At other times the blue granules of the zone are so abundant that the radial fibers cannot be observed in it, and they consequently appear to end at the periphery of the blue zone (Fig. 67). The round, red central body is occasionally seen to be surrounded by a clear space which again is bound by a definite red staining wall, around which the blue zone of granules is arranged (Figs. 67, 82). The central body may be very minute; and radial fibers, and, as it often appears,

radially arranged granules, immediately surround the small central body. The radial structures are then often limited by a broader or thinner zone of concentrically arranged fibers — the whole being conspicuously differentiated from all the rest of the cytoplasm (Pl. XIV, Fig. 53; Pl. XVI, Fig. 100). The outer concentrically arranged fibers may be absent, and the center may be a mass of granules surrounded by short, stiff, radial rods that penetrate unequally into the central granular mass. In the Biondi-Ehrlich stain, the central granules are yellow, while the radial rods are conspicuously red, the whole being definitely limited and sharply contrasted from the rest of the cytoplasm (Pl. XV, Fig. 80). In this case three bodies, connected by dense cytoplasmic fibers, are seen in the neighborhood of the spherical body; and from this the cytoplasmic fibers, arranged radially, can be seen to extend to the periphery of the egg, where they become continuous with the peripheral layer of fibrous protoplasm, thus rendering this portion of the egg peculiarly different from other portions of the same section. Instead of the radial rods and the three neighboring bodies, a comparatively large vesicular body, consisting of granules surrounded by a very distinct thin wall or membrane, has been observed. In this case only one body existed in its vicinity; and, as in the case above, the cytoplasmic fibers had a similar arrangement.

Occasionally a number of refractive bodies are to be seen at the apex of a cone of fibrous protoplasm, whose base is continuous with the peripheral protoplasm (Pl. XV, Fig. 90). It may also take the form of an oval sphere of interwoven fibers, enclosing in its meshes refractive bodies, and joined to the peripheral zone of fibrous protoplasm by a narrow stalk of similar fibrous protoplasm (Pl. XV, Fig. 84). Occasionally two refractive bodies removed from each other, but connected by a band of fibrous protoplasm, can be seen. From each of these refractive bodies, bundles of radial fibers extend far out into the cytoplasm, which is sharply contrasted from it. The body, when stained in Ehrlich's haematoxylin and eosin, is also seen to consist of a central sphere of yellow granules, which is surrounded by a bright-red, homogeneous, fibrous, protoplasmic

zone of considerable thickness, and having the form of a horseshoe (Pl. XV, Fig. 76). At the upper part of this fibrous protoplasm are imbedded several aggregations of blue granules; and, surrounding the whole, and slightly removed from it, in the red cytoplasm, is seen an irregular circle of these blue granules like a wreath, which, at the opening of the horseshoe-shaped protoplasm, forms an irregular mass of the blue granules. When stained in Ehrlich's haematoxylin alone, the body appears as an unstained spherical mass of fibrous protoplasm of considerable size, and definitely limited, around which are arranged, in a radial manner, numerous deep-blue granules apparently associated with a radial system of fibers proceeding from the body as a center (Pl. XVI, Fig. 96). Within such a body similar blue granules are found distributed, but most frequently aggregated into groups occupying vacuoles which with the blue granules appear like nuclei. Again it may appear as a conspicuous spherical body of the same fibrous protoplasm, which stains a deep red in erythrosin or acid fuchsin. It may enclose one or two nuclei-like bodies surrounded by a denser zone of the same fibrous protoplasm (Pl. XV, Figs. 64, 86). If the acid fuchsin be preceded with Ehrlich's haematoxylin, the central body (or bodies) is seen to contain the same blue granules resembling chromatin granules of nuclei (Figs. 86, 88). There may be several of these central bodies containing blue granules scattered irregularly between the fibers of a large felt-work of delicate protoplasmic fibers, as seen in Pl. XVI, Fig. 95. Occasionally the blue granules appear as highly refractive bodies lying between the fibers, that may have a comparatively regularly concentric arrangement (Pl. XIV, Fig. 61; Pl. XV, Fig. 72). Occasionally none of the granules can be observed (Fig. 69), but this may be due to the staining. Erythrosin, for instance, makes the whole body very conspicuous, but does not always differentiate the internal structures. The whole body may be relatively large, oval in form, and homogeneous in structure, having in this case, as in most cases, a system of surrounding radiations that extend far into the cytoplasm (Pl. XVI, Fig. 99). It may at times have the form of a spindle, with its longitudinal axis not exceeding the transverse axis, the

spindle appearance being due to the rather definite arrangement of the fibers with reference to two poles (Pl. XV, Fig. 65). At one of these poles appears a structure consisting of concentrically arranged microsomes, from which radiate in a somewhat irregular manner protoplasmic fibers.

In many cases the conspicuous, definitely limited spherical body, enclosing a central structure or nucleus, is seen to be surrounded by a definitely limited zone of protoplasm and granules that differ somewhat from the rest of the cytoplasm (Pl. XV, Fig. 71). The investing, interwoven, or concentrically arranged fibrous protoplasm, which so frequently surrounds the central nucleus or body, and which makes the whole so conspicuous when properly stained, is often limited in amount, or even apparently absent. The body may then be a spherical granular mass enclosed by a thin definite membrane. There may be two of these situated close together. They are made conspicuous by means of acid fuchsin or eosin (Pl. XV, Fig. 66).

As the egg grows the cytoplasm becomes more and more granular, and the body is less easily traced. The peculiar fibrous protoplasm, which often renders it so conspicuous, becomes less and less apparent. It often appears as a spherical mass of granules surrounded by a zone, in which they are few or entirely absent, and around which is another zone of similar granules. These granules being larger than those of the cytoplasm, and reacting differently towards stains, the body is still conspicuous (Pl. XVI, Fig. 102). With continued growth of the egg the body often attains to considerable dimensions. It may be a definitely spherical body surrounded by a clear ring, which, in connection with its deeper stain, sets it off conspicuously from the rest of the cytoplasm (Pl. XVI, Fig. 109). Or it may be irregular in outline, rather star shaped, granular, and may contain within it a spherical central body which alone often comes prominently into view (Pl. XVI, Fig. 114). This may be situated in the center of the egg and near to the germinal vesicle (Figs. 103, 104, 114). It varies, however, considerably in size; and it is at this stage difficult to differentiate it from the rest of the cytoplasm. Repeated attempts with a variety

of stains often bring it prominently into view even after all hopes of observing it have been abandoned.

Occasionally it may be more excentric. It is often very large, and consists of densely packed granules, which grade gradually into the surrounding protoplasm in which more conspicuous traces of the reticulated arrangement of the granules can be seen (Fig. 114). Here a zone of similar granules extends around the periphery of the egg, under the perivitelline layer of protoplasm. It is made conspicuous by means of the Biondi-Ehrlich stain, in which it takes a darker tinge than the rest of the cytoplasm. By means of Lyon's blue it also appears as a much darker body. By means of the double stain of erythrosin and cyanin it can be differentiated as a blue body, the rest of the cytoplasm being red (Fig. 104). The staining, however, must be applied with the greatest of care, and in a manner that can be learned only by repeated experiment. Having once acquired the necessary skill, however, it is a comparatively easy matter. From this it is not to be inferred that it is all a matter of staining, for the preliminary method of preserving is of even greater importance. Even with the best method of staining, it is to be observed only in the most perfectly preserved material. This is true not only of the later stages just described, but it is true also of the earlier stages.

In this description no attempt has been made to exhaust the subject, but merely to give the more prominent features of the body as it appears in the various phases of the growing egg.

All the figures in the plates, beginning with Fig. 48, Pl. XIV, are drawn with a Leitz camera, obj. 5, oc. 1-Leitz. Figs. 42–49 are drawn with a Leitz $\frac{1}{12}$ oil immersion.

Fig. 32 is one of the smallest eggs observed in the adult ovary, drawn with a camera, Leitz $\frac{1}{12}$ oil immersion.

## Interpretation and Summary.

After the last division of the oögonia to form a follicle, the centrosome, with its surrounding structures, persists in the cytoplasm. It has been observed in this case, not only during karyokinesis, but after the last reconstruction of the nucleus,

when the increased size of the cell and its nucleus shows it to be a growing egg (Pl. XIV, Figs. 34, 38–40). It consists, in this early stage, of two concentric circles of microsomes — a large outer circle and a smaller inner circle. In the center of the smaller inner circle is a granule, which at first hardly exceeds in size one of the microsomes of the surrounding circle. The microsomes of each circle appear to be connected by a less conspicuous fibrous substance; and from the microsomes of the inner circle to the microsomes of the outer circle radial fibers connecting these can be observed (Pl. XIV, Figs. 45–47). The minuteness of the inner circle and the body contained in it does not permit a determination, at this stage, of the presence or absence of radial fibers surrounding the central granule. The central granule, however, is present, though its minuteness often renders its detection difficult.

I do not hesitate to say that this is the centrosome of the dividing oögonia, and that the central granule, with its surrounding structure, corresponds very closely to that described by van Beneden in the dividing egg of Ascaris.

The centrosome and surrounding circles of microsomes, with their radial fibers, appear to be imbedded in a specially modified, more or less amorphous substance which, through the various effects of reagents and stains, renders the former obscure or even invisible. For the present I will adopt the term used by Boveri and call this imbedding substance archoplasm. This archoplasm is more conspicuous in some stains than in others; and for that reason the centrosome and surrounding structure may alone be distinctly visible; while with other stains the archoplasm appears prominent, often showing no internal structure. The former is true of such stains as picro-carmine (Fig. 47); the latter is often true of such stains as Lyon's blue and erythrosin and cyanin (Figs. 39, 40). A careful comparison of these different effects shows that at other stages both the radial system and circles of microsomes, as well as the archoplasm, are present.

Being a direct continuation of the centrosome of the dividing oögonia, it cannot be said to originate in the cytoplasm of the growing egg.

But is it derived from the young germinal vesicle? It exists before a nucleolus has made its appearance in the germinal vesicle. In this early stage the contents of the germinal vesicle and the granular cytoplasm stain a deep red in carmine or safranin. If one of these stains be properly associated with Lyon's blue, the centrosphere with its archoplasm stains a deep blue, the rest of the egg, both nucleus and cytoplasm, being bright red. The blue body is then observed as a conspicuous crescent-shaped structure partly enclosing the germinal vesicle. In the broadest portion of this crescent-shaped body the structure of the sphere and an enclosed centrosome, described above, can be seen (Figs. 42–47). The horns of the blue crescent appear to be due to an extension of the archoplasm of the sphere and an aggregation along the sides of the young germinal vesicle of the radial fibers belonging to the sphere. The blue crescent, although lying close to the germinal vesicle, is sharply differentiated from all parts of it, and is also at first sharply differentiated from the cytoplasm (Figs. 34, 38), although it later grades gradually into it. The red stain of the contents of the germinal vesicle and cytoplasm is due to the presence of granules of the nuclear network and the granules of the cytoplasm that are strongly affected by the carmine or safranin. The blue stain of the crescent-shaped sphere and archoplasm I would interpret as indicating an absence of the chromophilous granules. The nucleolus, when present, is also strongly affected by the carmine and safranin stains. When these stains alone are employed, the body remains obscure because of the absence of staining in that region; and one might easily, in such a case, be led to say that the body is not present. If, however, the carmine be followed with picric acid, the body comes into view as a yellowish body instead of the blue of the former double staining. There being present none of those granules which carmine so strongly affects, and which are the distinguishing features of chromatin of the nucleus, there is no ground on which to base the statement that this body originates from the germinal vesicle. Its form also will hardly admit the statement that it is a bud of the germinal vesicle. Furthermore, such a bud would necessarily contain

chromatin granules or else some substance capable of fixing the Lyon's blue more strongly than the carmine. The latter substance is not to be observed in the germinal vesicle, for no part of it takes the blue stain when carmine or safranin is associated with the Lyon's blue.

From these considerations, and others that will appear in another connection, it may be said that *the vitelline-body does not arise in the cytoplasm of the growing egg; neither does it arise as a bud of the germinal vesicle; nor as extruded chromatin, nor as migrating nucleoli. It contains no nuclear chromatin.*

In this connection, a few further considerations concerning Lyon's blue as a stain may be added. This stain not only differentiates the body under consideration in its earliest stages; but, in material preserved in suitable hardening reagents, it differentiates it conspicuously as long as safranin or carmine can be associated with it. This, however, ceases when the first period of growth is passed, since, after that period, these stains do not affect the granules of the cytoplasm. Yet, even after this, the body is made conspicuous by means of Lyon's blue used alone, because of its greater affinity for the stain and consequently deeper blue coloration. It may thus profitably be employed even in larger eggs of the second and third stages.

Even in those cases where nothing corresponding to the archoplasm appears, where the fibers of the cytoreticulum converge to a point as previously described, thus forming either a real, conspicuous aster, or a more irregular area with radial fibers, the center of this system is made conspicuous by the Lyon's blue, all the other parts of the egg being stained red by means of safranin. Where the body assumes the form of a large compact sphere of interwoven or concentric fibers, also, it is made prominent as a blue or green sphere standing out conspicuously from all the rest of the egg.

Although this stain, therefore, has a decided affinity for this body, I cannot regard it as a specific stain, for it shows also a decided affinity for the egg membrane after it has acquired several layers. Its general nature as a stain is evident further in those eggs belonging to the third stage, where it has been pointed out that numerous nuclei are found within the egg.

In such cases the carmine or safranin stains the chromatin granules of the nuclei ; and when this is followed with Lyon's blue, everything in the cytoplasm, except these red nuclei, stains a deep blue, making indeed handsome preparations. I should certainly hesitate, therefore, to regard everything as archoplasm in the sense in which Boveri used that term, which stains blue with this when combined with carmine and safranin. An examination of the plates of Miss Foot ('96), where the effect of this stain is extensively represented, would tend to increase rather than diminish such a reluctance. When associated with carmine or safranin, these are the specific stains. The value of the Lyon's blue lies in this, that it brings prominently into view those areas containing no chromophilous granules ; and for this purpose it is very convenient. In the first period of growth of the egg of Limulus it has been pointed out that both germinal vesicle and cytoplasm contain these chromophilous granules, this body alone being devoid of such granules. When eggs of Limulus are properly preserved, there are none of those irregular areas in the cytoplasm which Miss Foot has found in the egg of Allolobophora by means of this stain.

The vitelline-body having been shown to possess, in its earliest stages, all of the features of the centrosome and sphere, and to be, in fact, the centrosome of the dividing oögonia, it remains to show that the body found in the cytoplasm in later stages is the same centrosome. For this purpose the plates will afford better evidence than a labored description. The figures being drawn with a camera with the same magnifying power, show the body in the various stages of the growing egg. It can be seen that even in advanced stages of the egg the body often presents the fundamental features seen in the earliest stage, and often very nearly the features of a typical centrosome and sphere (Figs. 42–48, 67, 68, 82, 89, 114). These are characterized by the presence of a strongly refractive spherical body often surrounded by a clear zone, which again is surrounded by a zone of metaplasm (archoplasm Boveri) and provided with a system of radial fibers which can be seen to traverse the metaplasmic zone and to extend far out into

the cytoplasm. As these appearances have been described elsewhere, they need not be repeated here. The central refringent granule, staining deep red in acid fuchsin and surrounded by these radial fibers and metaplasmic zones containing the blue granules, is undoubtedly the centrosome; and it, with the surrounding structures, constitutes a real sphere. According to van Beneden ('87), the sphere consists of a central body (centrosome) surrounded by a clear zone (medullary zone), which again is surrounded by a granular zone (cortical zone). All of these conditions can be seen in the vitelline-body, in the egg of Limulus. According to Boveri ('89, '95), the centrosome is surrounded by a zone of archoplasm, which in some way grows out into the cytoplasm in the form of astral rays, which gradually replace the cytoreticulum. The vitelline-body presents the features of the sphere as defined by Boveri, and also the characteristics of a real aster (Figs. 43–46, 50–52, 54, 55, 57, 60).

But it is the unusual features which this body assumes that offer the greatest difficulties. Some of these are its excentric position, its large size, and the fantastic appearance which it often presents. The more common of these is the great increase in size of the central body (Pl. XV, Figs. 71, 77; Pl. XVI, Fig. 101), or the apparent absence of a definite central structure; the concentric arrangement of the fibers; their great increase or diminution; the often granular aspect of the body; the vesicular form which it sometimes assumes; and finally the combination in various ways of these different features. An attempt to account for these features will be made in the suggestions that are to be offered in the following chapters on some of the physiological problems of growth and metabolism.

These features are not foreign to the centrosome and sphere as these are now understood. I will only invite a comparison of some of the forms represented in the plates with the sphere in sperm cells of the salamander as figured by Rawitz ('95) and Meves ('94, '95), and in nerve cells as figured by Lenhossek ('95). Such a comparison will only serve to strengthen the conviction that the vitelline-body is indeed a sphere which not

only possesses the typical form of a centrosphere, the many forms of the real aster found in the dividing cells, in leucocytes, and in the fertilized egg of *Ascaris megalocephala*, but also the less typical forms observed in sperm cells as "Nebenkern," and in the resting ganglion cells.

*The vitelline-body in the ovarian egg of Limulus is genetically the centrosome and sphere of the dividing oögonia, and continues to be the centrosome and attraction sphere of the growing ovarian egg.*

That this centrosome and sphere may assume the form of the vitelline-body as originally described, seems evident from a comparison of Figs. 60–64, 70–72, 95 with the figures of Balbiani ('64), ('79), ('82), ('83), ('93); of v. Wittich ('49); Carus ('50); Schütz ('82); and Henking ('87); and becomes very evident when preparations of the ovary of Limulus and of the spider are directly compared.

The most important recent papers on the vitelline-body, Julin ('93), Mertens ('93), Balbiani ('93), and Henneguy ('93), also suggest strongly a probable relation of this body in other eggs to the centrosome and sphere.

*Position of the sphere.* — In the youngest eggs the sphere is always situated close to the germinal vesicle, as described above. It may remain in this position or it may become removed from the germinal vesicle so as to occupy a position midway between it and the periphery of the egg (Pl. XIV, Figs. 49, 56). At times it may even occupy a more excentric position (Fig. 70).

There appear to be three causes that can be assigned for this difference in position. First, a difference in the tension or contraction of the radial fibers; second, a difference in the local accumulation of the amorphous substances in the interfilar spaces; third, differences in actual growth of the cytoplasmic body.

The position of the metaplasm varies with reference to the central structure. It may spread out on either side of it, causing a density of the radial fibers lying close to the germinal vesicle, and thus causing the horns of the crescent (Pl. XIV, Fig. 47). From this position it may collect around the central

structure, greatly obscuring the latter, and causing the whole body to appear very conspicuous as a homogeneous solid body (Fig. 40). On the other hand, it may spread out in a circle surrounding the germinal vesicle (Fig. 48), and even become extended towards the point of attachment of the egg (Pl. XIII, Fig. 16). In that way it appears to form a channel by which food material is conveyed into the egg. In the vicinity of the germinal vesicle the food material is acted upon, or at least comes in contact with a clear fluid, perhaps karyolymph or nuclear sap, and becomes converted into conspicuous stainable granules. The metaplasm then, instead of collecting around the central structure, may move out into the cytoplasm, causing the fibers of the crescent to expand into the general cytoplasm; and the food granules surrounding the germinal vesicle may likewise be variously distributed, causing the inner zone surrounding the germinal vesicle either to entirely disappear or else to become hyaline and devoid of granules (Pl. XIV, Figs. 19, 24).

The variable disposition of these three elements — the cytolymph, as it may be called, the food granules, and the metaplasm — appears to be responsible for many of the variations, not only in the position, but also in the form of the vitelline-body. The position of the metaplasm with reference to this body appears to determine the direction of growth. If the metaplasm surrounds the central structure uniformly, the cytoplasm increases uniformly, and the body thus becomes gradually removed from the germinal vesicle. On the other hand, if the central structure lies close to the germinal vesicle, and the metaplasm on the distal side, the cytoplasm appears to increase in the direction of greatest amount of metaplasm; and if the metaplasm is wholly absent from the structure, as often appears to be the case (Figs. 50, 51, 55), the structure most frequently is found, even in later stages of the egg, to occupy the position which it formerly had (Pl. XVI, Fig. 104). In the absence of the metaplasm from the vicinity of the central structure, this latter does not appear as a conspicuous massive body, but as a fibrous framework of radial fibers with its refractive body in the center (Pl. XIV, Fig. 54).

It has been pointed out that eggs showing this feature of the body most conspicuously are chiefly those arising in later stages of growth of the parent organism, and therefore growing more slowly. This appears to be owing to the absence of nutritive material, which reveals itself in the very pronounced appearance of the reticulum (Pl. XIV, Fig. 32), which in eggs of the same size, arising earlier in the history of the animal, is often greatly obscured by the presence of an abundance of amorphous granules.

*Nature of the metaplasm.*—The yolk-nucleus (Pl. XIV, Fig. 27), I believe, can be considered as an early stage of the yolk, through which all yolk material passes on its way to become definite yolk spheres. It is often associated with the vitelline-body. Such a case seems to present itself here in the form of the metaplasm surrounding the centrosome and sphere.

The metaplasm consists of at least two kinds of granules. Some of these granules possess an affinity for haematoxylin, which is evident from their retaining this stain even when followed with such a powerful stain as acid fuchsin. In picro-carmine these granules show a reddish coloration in marked contrast with other portions of the granular metaplasm.

Careful examination of a large quantity of material showing these granules in connection with the vitelline-body and centrosome appears to show conclusively that they issue as little drops from the living substance of the cytoplasmic fibers and remain closely adherent to them. This appears to take place most freely when the fibrils are relaxed and in a state of rest. It is evident that where the fibers converge and are most densely packed, the number of these granules would be greatest. There is reason to suspect that these granules may be converted into yolk bodies, or be reabsorbed by the fiber so that no trace of them remains.

It has been shown that the granules in the cytoplasm of the first stages of the egg show an affinity for chromatin stains, and that later this affinity disappears as the definite yolk is formed. It has also been shown that these stainable granules are more abundant in those eggs which increase rapidly in size, and which may be supposed to be abundantly supplied with nutri-

ment. It has also been shown that in those young eggs which grow more slowly, being formed in a later period of the development of the parent organism, when growth is considerably retarded, and when therefore it may be supposed to be scantily supplied with nutriment, these granules are often comparatively scarce, often almost absent (Pl. XIV, Fig. 32). This fact suggests that the granules may have been used as food for the living substance, and that this is analogous to the appearance and disappearance of those granules which later appear in connection with the vitelline-body.

### Growth of the Cytoplasm.

The vitelline-body, as we have seen, is at first a central granule situated close to the germinal vesicle, surrounded by circles of large microsomes, probably connected by linin strands, and a system of fibers connecting the microsomes radially (Pl. XIV, Figs. 45, 46). In many cases this spherical structure is seen to become more granular, the granules being arranged concentrically, but yet closely packed. It may thus increase greatly in size (Pl. XIV, Fig. 56), while remaining refractive and apparently homogeneous, staining a bright red in acid fuchsin, and becoming surrounded by a zone of granules which retain the blue haematoxylin stain (Pl. XV, Fig. 78). In later stages this body may become still more enlarged, and consist of a large number of granules staining like the microsomes of the cytoreticulum and be surrounded by concentric or interwoven fibers, which again are surrounded by still another zone of granular substance (Pl. XV, Fig. 77). At the periphery of the central granular body the meshes between the granules may gradually increase so as to acquire the essential structure of the cytoplasm (Pl. XIV, Figs. 32, 46); or else a number of blue granules, like the chromatin of nuclei, are found within a felted mass of fibers which at the periphery passes gradually into the cytoreticulum (Pl. XV, Figs. 70, 76; Pl. XIV, Fig. 53).

In the absence of the blue granules the vitelline-body remains a compact mass consisting of closely packed microsomes

or fibrillae (Pl. XV, Fig. 69). *On the appearance of these granules, vacuoles arise; and the microsomes expand into the cytoreticulum* (Pl. XIV, Figs. 46, 53; Pl. XV, Figs. 72, 86). The formation of vacuoles and the resulting expansion may continue until the entire vitelline-body is reduced to a network (Fig. 32), and may seem to have entirely disappeared. The disappearance, however, is manifestly an illusion (Pl. XIV, Figs. 50, 51, 54, 55).

*It would seem that the attraction sphere, centrosome, and vitelline-body are the primitive basis or center of growth of the cytoplasm.*

The growth of the cytoplasm is greatest in the direction in which the blue granules are most abundant.

When the blue granules are unequally distributed around the central body, growth takes place unequally, leaving the body close to the germinal vesicle, or widely removed from it.

When the blue granules are equally distributed around the central body, growth takes place equally in all directions, and the body becomes the center of the cytoplasm.

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REFERENCE LETTERS.

| | | | |
|---|---|---|---|
| *m.* | muscle. | *y.n.* | yolk-nucleus. |
| *ov.t.* | ovarian tube. | *ca.* | carapace. |
| *an.* | anus. | *t.p.* | tunica propria. |
| *ov.* | ova. | *p.b.* | peripheral bodies. |
| *op.* | operculum. | *al.c.* | alimentary canal. |
| *ov.d.* | oviduct. | *p.c.* | peritoneal coat. |
| *g.o.* | genital opening. | *p.* | follicular pouch. |
| *fl.* | follicle. | *mpl.* | metaplasm. |
| *m.c.* | muscle coat. | *c.* | centrosome. |
| *e.s.* | egg stalk. | *ast.* | aster. |
| *g.v.* | germinal vesicle. | *n.* | nucleus. |
| *h.n.* | "Hauptnucleolus." | *ch.* | chorion. |
| *n.n.* | "Nebennucleolus." | *ncl.* | nucleolus. |
| *p.p.* | polar protoplasm. | *arch.* | archoplasm. |
| *y.z.* | yolk zone. | *sph.* | sphere. |
| *v.b.* | vitelline-body. | *b.g.* | blue granules. |
| *y.s.* | yolk secretion. | *p.m.* | polar mitosome. |
| *s.g.* | secretion granules. | *i.z.* | inner zone. |

## EXPLANATION OF PLATE XIII.

FIG. 1. Germinal vesicle with a crescent-shaped nucleolus, containing within it a reticulum resembling that of the germinal vesicle.

FIG. 2. A female Limulus with dorsal carapace removed, showing the netted ovary, the ovarian tubes on the right, *ov.t.*, being filled with eggs. The left side represents the condition in younger animals before eggs have been discharged into the ovarian tubes; *ov.*, young growing eggs; *an.*, anus; *op.*, operculum; *g.o.*, genital openings; *ca.*, carapace; *ov.t.*, ovarian tubes extending along the alimentary canal, *al.c.*, to the anus; *ov.d.*, terminal oviducts.

FIG. 3. Germinal vesicle showing "Nebennucleoli," and a body resembling these within the "Hauptnucleolus."

FIG. 4. Germinal vesicle showing a nucleolus having numerous internal bodies, one of which is about to be extruded.

FIG. 5. An amoeboid germinal vesicle containing a large nucleolus, in which there is a large vacuole containing nothing stainable.

FIG. 6. Germinal vesicle, containing a large hollow nucleolus in the form of a deeply staining shell. Within the nucleolus there is a granular network resembling the network of the germinal vesicle.

FIG. 7. Germinal vesicle showing diverticula, and containing a ring-shaped nucleolus containing within it a network resembling that of the germinal vesicle.

FIG. 8. Germinal vesicle with diverticula, and containing a nucleolus having a radial striation, a central granular mass, in which is imbedded a deeply staining, homogeneous spherical body — the endonucleolus.

FIG. 9. Germinal vesicle; a nucleolus in form of a deeply staining crescent containing a finely granular substance surrounding a vacuolated endonucleolus.

FIG. 10. An egg with its germinal vesicle containing a nucleolus, and also a radial arrangement of the chromatin network about a center resembling a centrosome.

FIG. 11. The nuclear pole ("Kernpol") of an egg about to be discharged from the follicle, showing the position of the germinal vesicle, and the spongy polar protoplasm spreading out under the egg membrane.

FIG. 12. Mature egg of the ovarian tube, showing an amoeboid remnant of the germinal vesicle, and its connection with a peripheral mass of protoplasm.

FIG. 13. Portion of an egg showing the formation of the egg membrane after the first layer has been formed; the orderly radial arrangement of the protoplasmic fibers previous to the hardening of the interfilar substance, showing that the radial striae of the chorion are due originally to protoplasmic fibers.

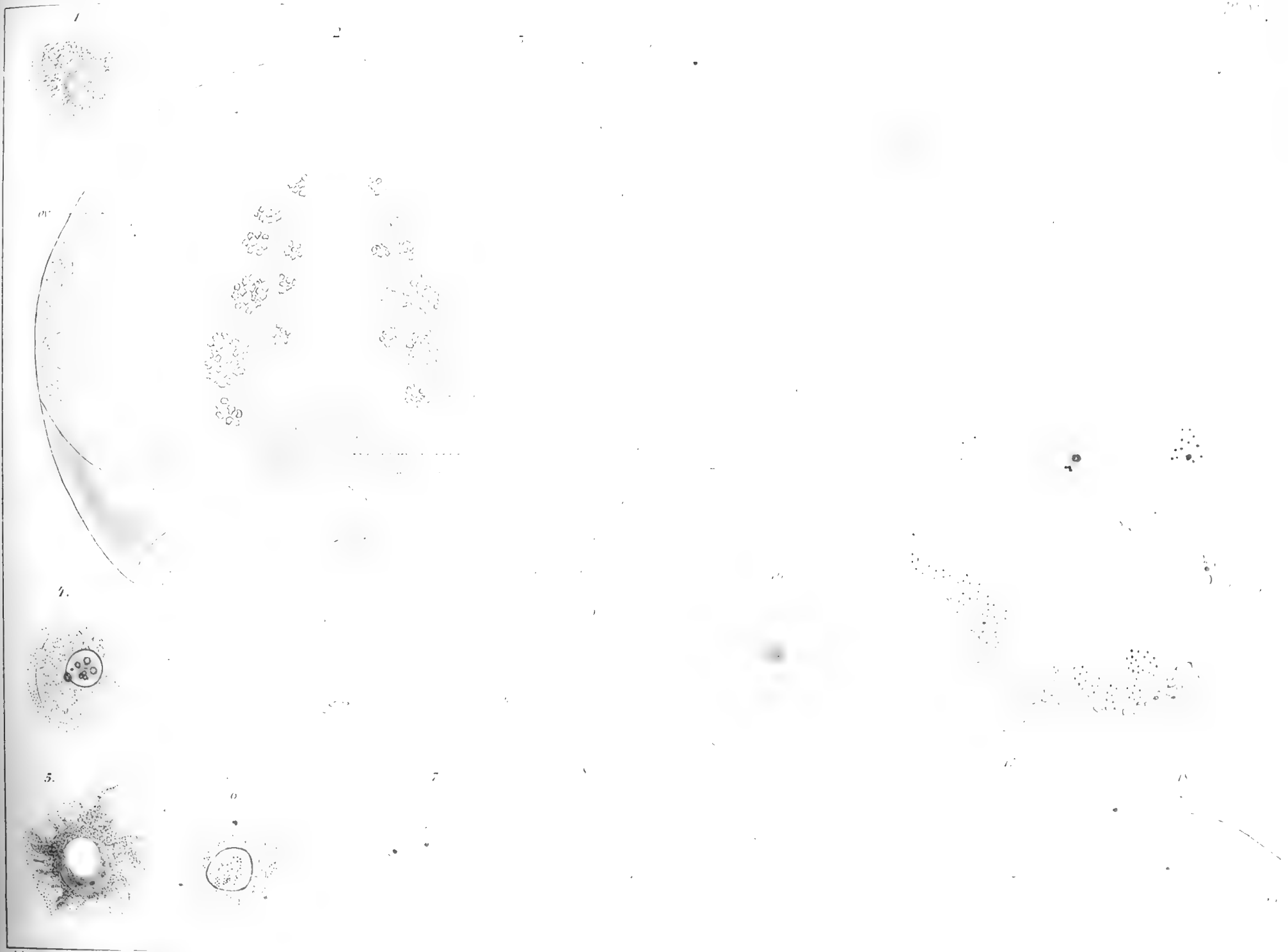
FIG. 14. Section of an egg about to be discharged into the ovarian tube, showing the movement of the germinal vesicle and its relation to the "Kernpol" area.

FIG. 15. Section of an adult ovarian tube, showing the folding of the germinal epithelium and with it the tunica propria, *t.p.*; the variable size and form of the epithelial cells; the formation of empty follicles, *f.*, by evagination through the fenestrae of the muscle coat, *m.c.*; the relation of the number and size of the eggs to the number of empty follicles; the position of the immature eggs with reference to the empty follicles and the point of attachment of the tube; the relation of this latter to the enclosing peritoneal coat. *p.c.*

FIG. 16. Portion of an ovarian tube taken from a living animal thirteen inches long, and examined in the normal fluids of the ovary, drawn with a camera; a sharply defined granular zone surrounding the germinal vesicle, as in *b.*, or extended on the proximal side to the point of attachment of the eggs, as in *c.*, or collected into a dense body in the cytoplasm, as in *a.*

FIG. 17. A portion of a mature egg in the ovarian tube, showing an opening in the chorion, and some distance below it the last remnant of the nucleus in the form of a spindle, the laminated structure of the chorion, and also its radial striations.

FIG. 18. Structure of the egg membranes; *p.t.*, primary tunic, corresponding to the tunica propria — the original egg membrane; the first egg membrane fully formed; the second having the form of radial protoplasmic fibers; the interfilar substance not yet hardened into the definite chorion. The primary tunic shows the bright dots — the original points of insertion of the radial fibers.



## EXPLANATION OF PLATE XIV.

FIG. 19. An egg showing an inner granular zone of the cytoplasm, and its connection with the stalk of the egg. The usual chromatin network of the germinal vesicle is wanting. No nucleolus is present. The tunica propria forming the basement membrane of the epithelium and the outer boundary of the stalk of the egg is continuous with the investing membrane of the egg; *m.c.*, muscle coat; *t.p.*, tunica propria; *y.z.*, internal yolk zone.

FIG. 20. An egg showing a hyaline inner yolk zone which is extended towards the point of attachment of the egg.

FIG. 21. An egg showing an inner yolk zone, *y.z.*, surrounding the germinal vesicle; in the dividing line between the inner and outer zone, the vitelline-body, *v.b.*, surrounded by a modified polar mitosome.

FIG. 22. The proximal portion of an egg showing the accumulation of yolk secretion, *y.s.*, at the base of the stalk of the egg, and the formation of the first layer of the egg membrane.

FIG. 23. An empty follicle serving as a yolk gland, showing the secretion products obscuring nuclei and cell boundaries; *s.g.*, secretion granules; *m.c.*, muscle coat.

FIG. 24. An egg showing an internal, hyaline protoplasmic zone surrounding the germinal vesicle as a uniform ring, *i.z.*; the germinal vesicle with diverticula and the nucleolus containing a vacuole.

FIG. 25. An egg showing the peripheral bodies, *p.b.*, and their gradual fusion into the first layer of the egg membrane or chorion, *ch.* Low magnifying power.

FIG. 26. Small egg with a germinal vesicle containing two "Hauptnucleoli," *h.n.*, each extruding a "Nebennucleolus," *n.n.*

FIG. 27. The proximal portion of an egg, showing deeply stainable bodies resembling nuclei — the yolk-nucleus, *y.n.* The basement membrane of the stalk of the egg is seen to be continuous with the primary egg-tunic, *t.p.*

FIG. 28. Tangential section of an egg, showing the form and distribution of the peripheral bodies, *p.b.*, and the round bright dots or pores with which they are pierced.

FIG. 29. Small egg showing the internal granular zone, *y.z.*, in the form of a ring surrounding the germinal vesicle, the latter containing no true chromatin reticulum and no nucleolus.

FIG. 30. An egg in the third stage, filled with nuclei, and partly surrounded by nucleated granular cells; no germinal vesicle.

FIG. 31. An egg (similar to Fig. 30) undergoing regressive metamorphosis; no germinal vesicle; proximal portion of the yolk filled with nuclei and a hyaline protoplasm; egg surrounded by a false follicle epithelium of granular cells, as in Fig. 30; chorion folded, distorted, and pierced in various ways.

FIG. 32. A small egg of an adult ovary (magnified $\frac{1}{12}$ oil immersion), showing a very distinct cytoreticulum, and a sphere in the form of a central dense network with very distinct radial fibers, many of which appear to be continuous with the general cytoreticulum; the cytoreticulum apparently continuous with the nuclear network; metaplasm (archoplasm) scant or absent.

FIG. 33. Section of an ovarian tube of a young Limulus (Leitz, oc. 1, obj. 7), showing a group of cells undergoing the preliminary phases of karyokinesis previous to the formation of a growing oöcyte.

FIG. 34. Young Limulus, six-inch ; transverse section of ovarian tube showing oögonia ; the spireme of karyokinesis, and a growing oöcyte. The young germinal vesicle contains a nucleolus ; in the cytoplasm, close to the germinal vesicle, the crescent-shaped archoplasm. Method : nitro-picro-sulph., lithium-carmine, Lyon's blue. Archoplasm alone a deep blue.

FIG. 35. Six-inch animal ; longitudinal section ovarian tube ; chromatin thread — spireme of karyokinesis. Method: Merkel's fluid, Heidenhain's iron-haematoxylin, oc. 1, obj. 7; *t.p.*, tunica propria ; *p.c.*, peritoneal coat.

FIG. 36. Six-inch animal ; transverse section ovarian tube, cut obliquely ; metaphase and anaphase of karyokinesis ; centrosome at the pole of one spindle.

FIG. 37. Seven-inch animal ; transverse section ovarian tube ; oögonia in karyokinesis ; spindles ; equatorial plate. Method: Merkel's fluid, Heidenhain's iron-haematoxylin.

FIG. 38. Seven-inch animal ; transverse section of ovarian tube ; oögonia ; growing oöcyte with blue archoplasm. Method (3) (see method), Lyon's blue, lithium-carmine.

FIG. 39. Seven-inch animal ; ovarian tube ; oögonia ; growing oöcyte forming a diverticulum, and surrounded by the tunica propria and by the nucleated peritoneal mantle. Blue archoplasm conspicuous.

FIG. 40. Seven-inch animal ; oblique section of ovarian tube, showing three growing oöcytes, one of which has formed a diverticulum, and remains attached only by a narrow stalk ; enclosed first by the tunica propria, and second by the peritoneal nucleated coat. Method : No. 3, Lyon's blue and lithium-carmine. Blue archoplasm very distinct.

FIG. 41. Thirteen-inch animal; transverse section of ovarian tube, showing the relation of the ovarian tube, lined with an epithelium, which is bounded by the tunica propria, *t.p.*, to the investing mantle or peritoneal coat, *p.c.* The point of attachment of the tube is seen to be also the point of origin of the eggs. From this point the eggs are seen to increase in size, regularly, to the point opposite where the largest egg is found.

FIGS. 42–47. Growing oöcytes from ovary of animal seven inches. All drawn with Leitz camera, $\frac{1}{12}$ oil immersion, and showing the centrosome and archoplasm, and the relation of these to the cytoreticulum. Method (3).

FIG. 42. Eosin and nigrosin.

FIGS. 43, 44. Lithium-carmine and Lyon's blue; archoplasm and centrosome, blue; everything else, red.

FIGS. 45, 46. Delafield's haematoxylin and picric acid ; centrosome and sphere, yellow ; everything else, blue ; sphere, very distinct.

FIG. 47. Weigert's picro-carmine ; archoplasmic sphere ; cytoreticulum and central radial structure very distinct.

FIG. 48. One of the smallest eggs from an ovarian tube, like that shown in Fig. 41. A distinct centrosome in the center of a light area, in the widest portion of the metaplasmic zone surrounding the germinal vesicle. In the latter, a prominent nucleolus.

FIGS. 49–104, 109, 114. The entire series of figures from Fig. 49 is drawn on the same scale, Leitz camera, oc. 1, obj. 5.

FIG. 49. One of the smallest eggs observed in the adult animal, showing a germinal vesicle, *g.v.*, with a granular nucleolus; a very distinct blue sphere, *sph.*, with bright-red central granules. Method: Merkel's fluid, safranin, and Lyon's blue. Sphere, *sph.*, alone bright blue ; everything else, red.

FIG. 50. One of the smallest eggs of an old animal having many empty follicles. Cytoreticulum and aster, *ast.*, very distinct. Metaplasmic granules or archoplasm, very scarce or absent. Method: Merkel's fluid, Biondi-Ehrlich stain.

FIG. 51. Similar to Fig. 50; egg a little larger; metaplasmic granules more abundant. Method same as Fig. 50.

FIG. 52. A very distinct, sharply limited aster, *ast.*; *g.v.*, germinal vesicle; *ncl.*, nucleolus, containing a central vacuole.

FIG. 53. Egg from adult animal, showing a blue sphere, *sph.*, consisting of a central body, centrosome, surrounded by radial astral rays that become lost in a zone of archoplasmic granules, which again is surrounded by compacted fibers that merge gradually into the cytoplasmic reticulum.

FIG. 54. Egg showing a distinct cytoreticulum, in which there is a conspicuous aster, *ast.* This is farther removed from the germinal vesicle, *g.v.*, than the similar structures seen in Figs. 50, 51.

FIG. 55. Section of an egg showing position of aster in a plane at right angles to the primary egg axis.

FIG. 56. Section of an egg preserved in Flemming's fluid and stained in acid fuchsin. A large, bright-red body, *c.*, in the cytoplasm, whose reticulum is arranged radially around the centrosome and sphere.

FIG. 57. Section of an egg showing a central body or centrosome, and a conspicuous aster, *ast.* Method: corrosive acetic, haematoxylin, and acid fuchsin.

FIG. 58. Section of an egg showing sphere, *sph.*, with two central structures surrounded by archoplasm. Method: safranin and Lyon's blue. The sphere alone, deep blue; the rest of the cytoplasm and the germinal vesicle, red.

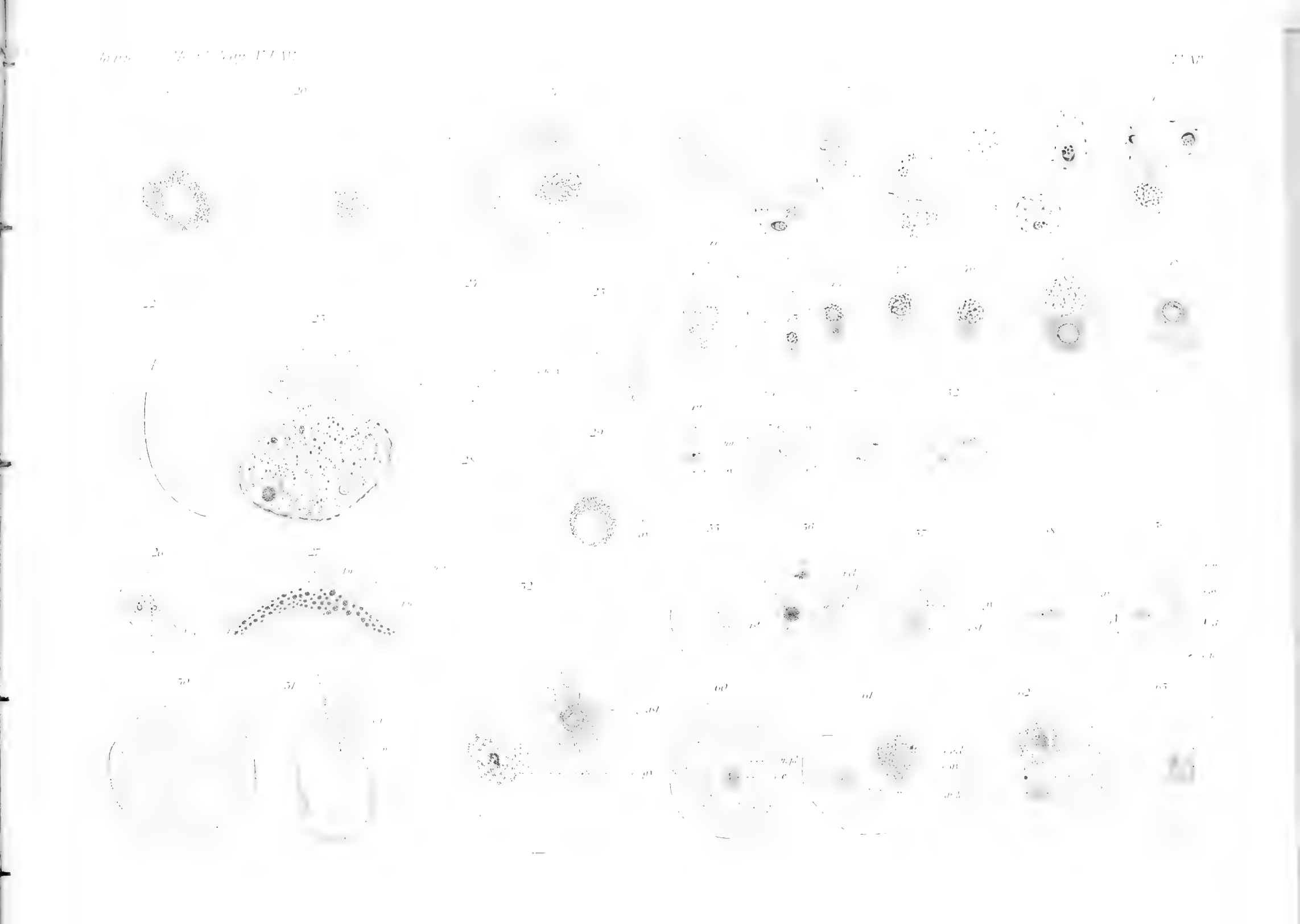
FIG. 59. Section of egg showing germinal vesicle, *g.v.*; nucleolus, *ncl.*; peripheral bodies, *p.b.*; and a centrosome and sphere, *c.*, connected with a fibrous polar protoplasm, or polar mitosome, *p.m.*

FIG. 60. Section of an egg showing a large granular central body, *c.*, a zone of fibrous protoplasm, and a zone of granular metaplasm (archoplasm), *m.pl.*, from which radiate many cytoplasmic fibers or astral rays.

FIG. 61. Section of egg showing archoplasm, *arch.*, containing many vacuoles and a central granular body, the centrosphere; *g.v.*, germinal vesicle; *ncl.*, nucleolus.

FIG. 62. Section of egg showing a conspicuous sphere, *sph.*, containing a central body, centrosome (*c.*), and surrounded by a zone of blue granules, *b.g.* The sphere, *sph.*, red. At the proximal pole, the modified polar mitosome.

FIG. 63. Section of egg showing a conspicuous vitelline-body, *v.b.* The peripheral protoplasmic layer is seen to extend into the central granular mass, and to thus divide the latter into three portions.



## EXPLANATION OF PLATE XV.

FIG. 64. Section of egg showing a conspicuous, deep-red, homogeneous vitelline-body or sphere, containing central granular bodies or centrioles.

FIG. 65. A deep-red vitelline-body, whose fibers are arranged with reference to two poles, at one of which there is a sphere with archoplasm and radial fibers. Method: Merkel's fluid, eosin.

FIG. 66. Section of egg showing peripheral bodies, a germinal vesicle with nucleolus; in the cytoplasm, archoplasmic sphere containing two large spherical central bodies.

FIG. 67. Section of egg showing peripheral bodies, a conspicuous red centrosome, surrounded by a clear zone, which again is surrounded by a zone of blue granules, and this again surrounded by archoplasm. Radial fibers proceeding from this can be traced throughout the entire cytoplasm. Method: Merkel's fluid, Ehrlich's haematoxylin and eosin.

FIG. 68. Section of egg showing germinal vesicle, containing a nucleolus, in which is seen a central spherical body, the endonucleolus. In the cytoplasm is a conspicuous deep-red centrosome, surrounded by a zone of blue granules, and this again by a system of red astral rays extending to the periphery of the egg. Method: Merkel's fluid, Ehrlich's haematoxylin, and acid fuchsin.

FIG. 69. Section of egg preserved in Hermann's fluid, showing a conspicuous homogeneous vitelline-body with indications of astral rays proceeding from it. Method: Hermann's fluid, acid fuchsin.

FIG. 70. Section of egg showing germinal vesicle and nucleolus; and in the cytoplasm a conspicuous vitelline-body containing granular vacuoles, a system of astral rays, and numerous blue granules. Method: Merkel's fluid, Ehrlich's haematoxylin, and acid fuchsin.

FIG. 71. Section of egg showing germinal vesicle containing a large "Hauptnucleolus" and small "Nebennucleolus." Close to the germinal vesicle, a sharply defined sphere with indistinct granular rays, and a large granular central body. Method: Merkel's fluid, eosin.

FIG. 72. Section of egg with germinal vesicle containing nucleolus; in the cytoplasm a deeply staining sphere containing numerous granular vacuoles.

FIG. 73. Section of egg with germinal vesicle, nucleolus, a prominent vitelline-body or sphere, consisting of a sharply defined granular body, partly surrounded by a zone of fibrous archoplasm. Method: Merkel's fluid, haematoxylin, and eosin.

FIG. 74. Section of egg with germinal vesicle, a "Hauptnucleolus," and a "Nebennucleolus." In the cytoplasm a conspicuous sphere with a central lighter granular body, surrounded by a broad zone of deeply staining archoplasm. Method: Kleinenberg's picro-sulphuric, haematoxylin, and acid fuchsin.

FIG. 75. Section showing the same as above. The sphere, deep blue, shows central body, centrosome, and a somewhat regular arrangement of radial fibers to a peripheral concentric protoplasmic zone. Method: Merkel's fluid, safranin, and Lyon's blue.

FIG. 76. Section with germinal vesicle, nucleolus; in the cytoplasm a vitelline-body, consisting of a central granular body which is partly surrounded by a horse-shoe-shaped archoplasmic zone. In the outer portion of this red archoplasm are

three vacuoles filled with blue granules. The entire body is surrounded by a zone of blue granules, which are more numerous at the opening of the horseshoe-shaped archoplasm. Method: Merkel's fluid, Ehrlich's haematoxylin, and eosin.

FIG. 77. Section showing peripheral bodies, a central sphere with astral rays. The center of the sphere consists of closely packed blue granules, and this is surrounded by a thick dense red limiting membrane. Method: Merkel's fluid, erythrosin, and cyanin.

FIG. 78. Section showing a proximal polar mitosome, a conspicuous vitelline-body, staining red, and surrounded by a zone of blue granules. Similar granules are seen also at the pole opposite the stalk of the egg.

FIG. 79. Section of egg showing germinal vesicle with a prominent "Hauptnucleolus," and an extruded "Nebennucleolus"; peripheral bodies at the periphery of the egg; in the cytoplasm, an archoplasmic sphere, containing two central structures. Method: Merkel's fluid, safranin, and Lyon's blue. The sphere alone, blue or greenish; everything else, red. A deep-red circle at the proximal pole.

FIG. 80. Section showing a vitelline-body, consisting of a central, irregular, granular mass, from which radiate straight fibers, which at a certain distance from the central body are again limited by the granules of the cytoplasm. A peculiarly modified fibrous protoplasm exists in the neighborhood of the body, and this is connected with three small refractive bodies imbedded in a strand of protoplasm. Method: Merkel's fluid, Biondi-Ehrlich.

FIG. 81. Section showing germinal vesicle with vacuolated nucleolus; a vitelline-body or sphere, consisting of fibrous protoplasm, containing two central granular bodies. Method: Merkel's fluid, erythrosin, and cyanin.

FIG. 82. Section showing germinal vesicle, nucleolus; in the cytoplasm a large, sharply defined, deep-red centrosome, surrounded by two zones of archoplasm and astral rays, the latter on one side being modified into a conspicuous polar mitosome. Method: Merkel's fluid, haematoxylin, and acid fuchsin.

FIG. 83. Section showing germinal vesicle with vacuolated nucleolus; in the cytoplasm a large, homogeneous sphere containing vacuoles with blue granules, partly surrounded by groups of blue granules resembling nuclei. Method: Merkel's fluid, Ehrlich's haematoxylin. The homogeneous part of sphere, unstained.

FIG. 84. Section showing germinal vesicle, a vacuolated nucleolus; in the cytoplasm a large oval fibrous vitelline-body, containing granules, and connected with the periphery of the egg by a modified fibrous protoplasm resembling that of the vitelline-body. Method: Merkel's fluid, Biondi-Ehrlich stain.

FIG. 85. Section showing germinal vesicle with diverticula; a large vesicular nucleolus, containing granules, and in the cytoplasm a large, finely granular sphere, which is partly surrounded by a dense zone of granular archoplasm. Method: corrosive-acetic, haematoxylin, and picric acid.

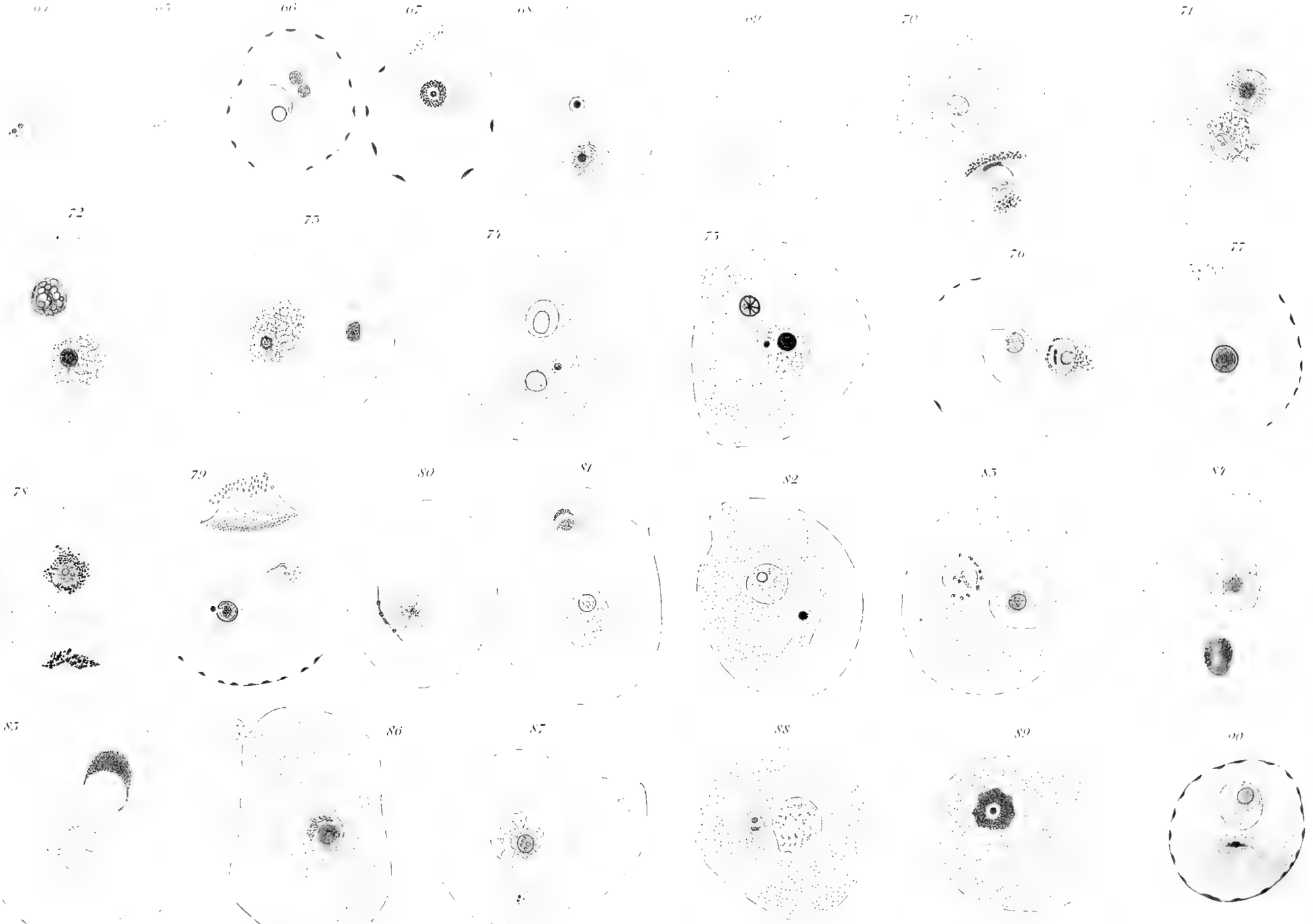
FIG. 86. Section showing a conspicuous, deep-red, fibrous vitelline-body (sphere), containing two central vacuoles with blue granules, and numerous peripheral vacuoles with similar blue granules, the whole being surrounded by a zone of larger metaplasmic granules and astral radiations. Method: Merkel's fluid, Ehrlich's haematoxylin, acid fuchsin.

FIG. 87. Section showing germinal vesicle, a vacuolated nucleolus, and in the cytoplasm one or two centrosomes surrounded by conspicuous silken astral rays that are not sharply limited. Method: corrosive-acetic, haematoxylin.

FIG. 88. Section showing germinal vesicle, a pale "Nebennucleolus"; in the cytoplasm a sharply defined, deep-red, fibrous sphere, containing two centrioles. Method: Merkel's fluid, erythrosin, and cyanin.

FIG. 89. Section showing sphere with a distinct centrosome in a lighter space, which is surrounded by a broad zone of archoplasm and astral rays extending throughout the egg. Method: Merkel's fluid, Weigert's picro-carmine.

FIG. 90. Section showing germinal vesicle, nucleolus; in the cytoplasm a granular elongated body at the apex of a fibrous cone of protoplasm. Around the periphery are numerous peripheral bodies. Method: Merkel's fluid, Biondi-Ehrlich.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## EXPLANATION OF PLATE XVI.

FIG. 91. Section showing germinal vesicle, nucleolus; in the cytoplasm a granular yolk-nucleus at the proximal pole; connected with it, a proximal polar mitosome. Method: Merkel's fluid, Biondi-Ehrlich.

FIG. 92. Section showing germinal vesicle; in the cytoplasm a conspicuous aster with a large, deeply staining granular centrosome. Method: Merkel's fluid, erythrosin, and cyanin.

FIG. 93. Section showing germinal vesicle with nucleolus; peripheral bodies at the boundary of the egg; a peculiar vitelline-body consisting of a spherical mass of fibrous protoplasm, connected by a stalk with a finely striated protoplasm at the pole opposite the stalk of the egg; a few refractive granules, near the stalk of the spherical body.

FIG. 94. Section showing germinal vesicle with a large vacuolated nucleolus; in the cytoplasm a large homogeneous fibrous vitelline-body, having a mass of granules at one pole.

FIG. 95. Section showing germinal vesicle with diverticulum, and a conspicuous nucleolus; a large vitelline-body, having numerous vacuoles, containing the blue granules and a central structure, probably the centrosome. Method: Merkel's fluid, erythrosin, and cyanin.

FIG. 96. Section showing vitelline-body with astral rays, and surrounded by groups of blue granules arranged radially; within the body, also, a group of blue granules. The fibrous portion unstained. Method: Merkel's fluid, Ehrlich's haematoxylin.

FIG. 97. Section showing vitelline-body as a large granular central body, surrounded by a zone of archoplasm and a conspicuous system of radial fibers, which extend to the periphery of the egg, and is especially pronounced at the pole opposite the point of attachment of the egg. Method as above.

FIG. 98. Section showing vitelline-body with several granular central areas. Method as above.

FIG. 99. Section showing germinal vesicle with a large vacuolated nucleolus; in the cytoplasm a large, homogeneous sphere staining deeply, and surrounded by radial fibers. Method: Merkel's fluid, erythrosin, and cyanin.

FIG. 100. Section showing germinal vesicle, containing vacuolated nucleolus; in the cytoplasm a distinct sphere, containing a central granule, centrosome, with radial fibers and granules, which is again bounded by layers of fibrous protoplasm concentrically arranged; the body, surrounded by a zone of large, stainable granules; on the proximal side a modified polar mitosome. Method: Merkel's fluid, Biondi-Ehrlich.

FIG. 101. Section showing central sphere, consisting of a large, spherical, central body staining blue, and composed of blue granules, and another zone of concentrically arranged fibrous protoplasm; indications of astral radiations. Method: Merkel's fluid, Ehrlich's haematoxylin, and acid fuchsin.

FIG. 102. Section showing germinal vesicle with large central nucleolus; in the cytoplasm a conspicuous sphere, consisting of large granules, a central granular body being separated from an outer granular zone by a light ring nearly free from granules. Method: Merkel's fluid and Biondi-Ehrlich stain.

FIG. 103. Section showing germinal vesicle with a large nucleolus. At one pole of the germinal vesicle in the cytoplasm there is an area which shows a faint

radial striation and also a faint concentric striation. It is less granular than the rest of the cytoplasm, and is no doubt the sphere. It resembles the condition seen in Fig. 104, but is less conspicuous.

FIG. 104. Section drawn with a camera with the same magnifying power as the preceding figures, and showing the sphere in connection with the germinal vesicle. The latter contains a large nucleolus. The hyaline protoplasm, constituting the "Kernpol" area, is seen near the point of attachment of the egg. Method: platinum chloride, erythrosin, and cyanin.

FIG. 105. Germinal vesicle with diverticula containing the nuclear network and "Nebennucleoli"; also a large "Hauptnucleolus," showing an opening into the central cavity.

FIGS. 106, 108, 115–118. Nucleoli taken from the living egg of an animal thirteen inches long. In Figs. 115, 116 two "Hauptnucleoli," one large and one small, are present. In Fig. 118 the two are closely united.

FIG. 107. Germinal vesicle with a nucleolus containing within it a network resembling that of the germinal vesicle.

FIG. 109. Section showing a condition of the sphere similar to Fig. 104, cut at right angles to the principal egg axis. Method: same as above.

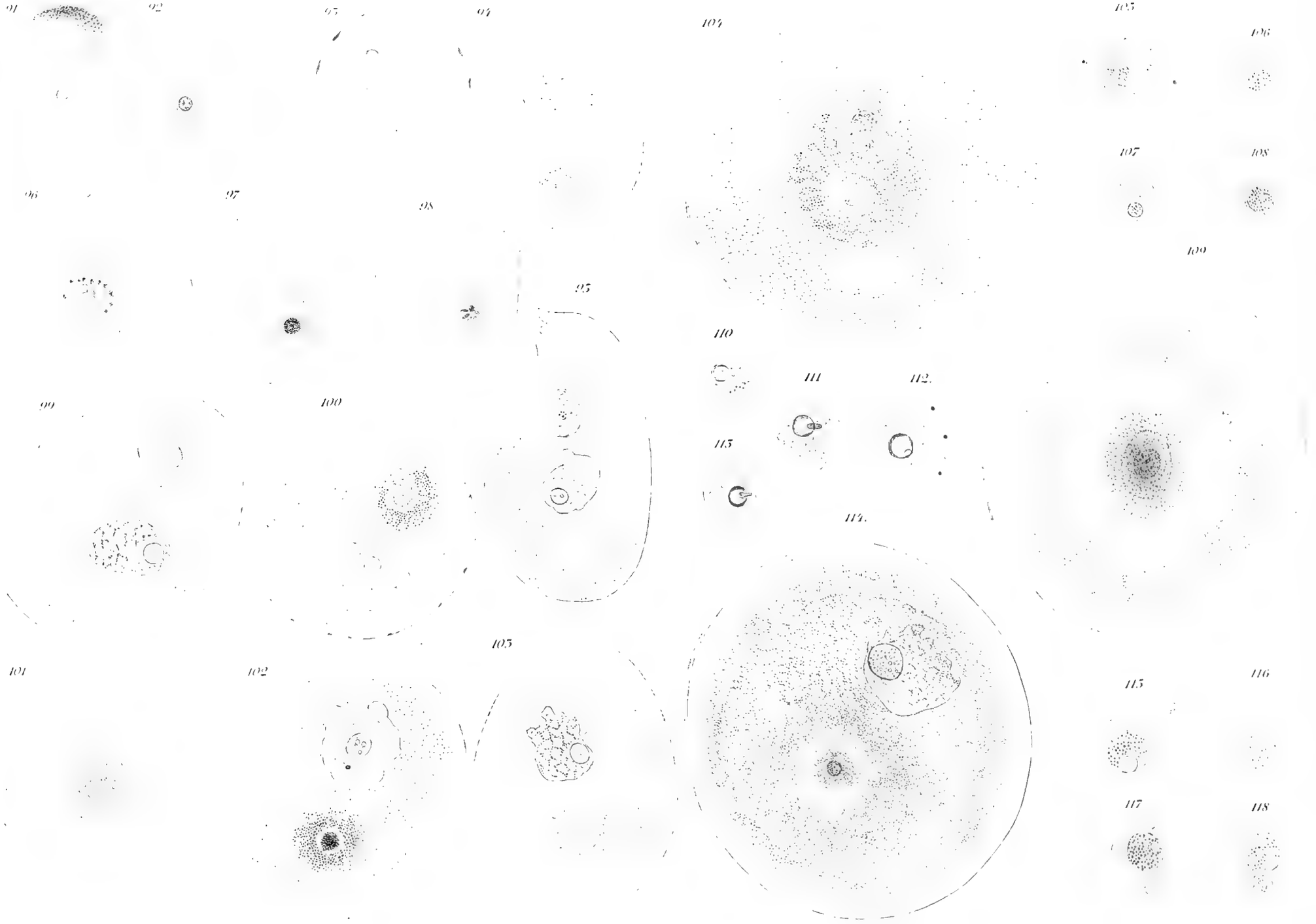
FIG. 110. Germinal vesicle containing a "Hauptnucleolus," from which a "Nebennucleolus" has been extruded.

FIG. 111. Germinal vesicle containing a nucleolus with the "Nebennucleolus" partly extruded.

FIG. 112. Germinal vesicle with a "Nebennucleolus" partly extruded. In one diverticulum of the germinal vesicle is seen a pale "Nebennucleolus," and in the cytoplasm near by are two similar bodies.

FIG. 113. Germinal vesicle with a "Hauptnucleolus," from which a "Nebennucleolus" is partly extruded.

FIG. 114. Section showing the germinal vesicle containing a large nucleolus; and in the cytoplasm a large sphere, in which a radial striation can be made out. The center contains a large clear area, in which a centrosome is difficult to find, when the other portions of the sphere are made prominent. In the present figure the central body with its centrosome and archoplasmic zone was taken from another section of an egg of exactly the same size as the present one, as in that case the central portion was better preserved than the peripheral portion. Method: platinum chloride and Biondi-Ehrlich stain. Excellently preserved.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# THE LATERAL LINE SYSTEM OF BATRACHUS TAU.

CORNELIA M. CLAPP.

CONTENTS.



## Introduction.

In a recent contribution to the "Skin and Cutaneous Sense Organs of Teleosts," Leydig (1) says: "Every one who has worked in this field shares the conviction that there is need of the coöperating participation of many observers before any conclusive presentation of the subject is possible."

As the discoverer of the true nature of the so-called "lateral line" of fishes, Leydig's words have weight, when, after nearly fifty years of investigation, he is obliged to confess that "the points still obscure outnumber by far those well ascertained."

Kupffer maintains that we are still on the very threshold of a history of the development of the peripheral nervous system. It is therefore the task of the investigator to furnish data for the future work of generalization.

In the following pages an attempt has been made to describe the lateral line system of the toadfish, both in the adult and developmental stages, for, as Mr. Allis (2) has well said, the "purely descriptive part of the subject" has been too much neglected.

Frequent reference will be made to the conditions existing in Amia, and the nomenclature employed by Allis will be adopted.

Ryder (3) describes the appearance of the lateral line organs in the young toadfish at the time of the formation of canals on the head, and speaks of the lines of free organs on the body as *canals!* It is evident that his observations, though in many ways valuable, were incomplete. So far as I am aware, this preliminary "notice" contains all we have on the subject of the lateral line system of the toadfish.

In Jordan's "Synopsis of Fishes of North America" (4) the only mention of this system is the statement that in Batrachus there is "*no lateral line*, nor conspicuous pores."

My study of the lateral line system of Batrachus was begun in the summer of 1888, under the direction of Prof. C. O. Whitman, at the Marine Biological Laboratory of Woods Holl, Mass., and completed at the University of Chicago.

I wish to express my deep feeling of obligation to Professor Whitman for the interest he has taken in the supervision of my work; and for the many courtesies and suggestions received from instructors and associates, I wish here to make acknowledgment.

For assistance in obtaining material at Woods Holl I am greatly indebted to Mr. G. M. Gray, the Collector of the Marine Biological Laboratory; for specimens of Acanthias I wish to thank Dr. A. D. Mead and Prof. A. D. Morrill.

The drawings for this paper were made after my sketches by the following draughtsmen at the Marine Biological Laboratory:

Figs. 1–3 and 7–11 were made by Mr. Crosby.
" 4–6 " " " Mr. Tokano.
" 12 " " " Mr. John Walton.
" 22, 23, 24 " " " Mr. Hayashi.

## I. Adult Form.

### *General Description.*

There is something singularly grotesque in the appearance of the toadfish; and, as its name would imply, there is a superficial resemblance to the familiar batrachian. The sluggish disposition, the mottled brown and gray of the wrinkled, scaleless skin, the depressed head and toadish eyes do not suggest the typical teleost. The young fish also are tadpole-like in their form and motions.

From Pl. XVII, Figs. 1–3, it will be seen that there are quite conspicuous projections of the skin on the head. Besides the paired flaps found in connection with the sense organs, there are other single, often longer projections to be found, which become laciniated in the older fish. These are especially prominent about the mouth, fringing the margin of the lower mandible and opercular regions, while over each eye rises a broad conspicuous flap, giving an owl-like facial expression. The goosefish (Lophius) and the sea raven (Hemitripterus) also possess these somewhat ornamental appendages about the mouth. The function of these skinny tentacles seems evidently to be for protection, as they strikingly resemble both in color and form the seaweed (fucus) that abounds near their favorite haunts.

The toadfish frequents the shallow water of bays and inlets of the sea, ranging on the Atlantic coast from Cape Cod to Florida.

It is abundant at Woods Holl, Mass., and is easily obtainable in the month of June, during the spawning season. At this time the fish resort in pairs to large stones, usually near low watermark, and scooping out a cavity beneath, remain for days in their retreat. The toadfish of the Eel pond near the laboratory seem to prefer the *débris* of civilization to the excavation beneath the rock; for example, tin cans, old boots, broken jugs, etc. After depositing the eggs, the female departs, while the male remains to guard the nest.

The young fish do not "attach themselves by a ventral disc which soon disappears," as has been supposed, but at the time

of oviposition each egg is securely glued to the rock by means of a secretion on the egg membrane at the pole of the egg opposite the micropyle.

After hatching, the embryo fishes still remain attached to the rock by the adhesion of the yolk sac to the inside of the egg membrane over the disc area, until the yolk material has been entirely absorbed — a period of three or four weeks.

The largest toadfish seldom reaches a length of more than twelve inches.

Dr. Goode (5) gives the following facts about the toadfish: "In general appearance it resembles a sculpin. It possesses the power of changing its color to lighter or darker shades when exposed to light in shallow vessels with dark or light colored bottoms. It probably becomes torpid in winter in the more northern regions, is very hardy, and utters a loud croaking sound when handled."

In Storer's description of Batrachus tau one finds certain statements which are hardly correct. For example, he speaks of the eggs as being "not larger than very small shot," as "increasing in size" after deposition, also as adhering by a "disc acting as a sucker," and finally he says of the fish which remains to guard the eggs, that "it is in all cases the mother of the young ones."

### *Topography of the Lateral Line System.*

1. *Infraorbital line.* — The first six organs of this line are found on a semicircular fold of the skin, anterior to the nasal tube (Pl. XVII, Fig. 2). These organs constitute the antorbital portion of the infraorbital line. They are free organs protected by a pair of flaps of the skin, representing in their position the sides of a canal. Each organ occupies a depression in the skin, and on opposite sides are developed the pointed flaps which arch over this depression, the tips of the flaps almost meeting over the center of the organ (Fig. 1).

There is no anterior commissure between the infraorbital lines of the two sides of the head as seen in Amia.

At a point midway between the anterior and posterior nares the infraorbital line branches. One division extending along the border of the maxillary may therefore be called the maxillary branch, the other being the suborbital portion of the main line (Fig. 1). There are seven organs in this maxillary branch, five being free organs and two enclosed in a short canal (Pl. XX, Fig. 22). The suborbital portion consists of eight free organs, bordering the lower half of the orbit (Figs. 22 and 23).

At the outer angle of the eye there are two free organs (9, 10) continuing the line of the infraorbital and corresponding to the otic portion (15, 16) as seen in Amia. (Compare Pl. XX, Figs. 21 and 22.) In the temporal portion of the line there is a single organ (11) enclosed in a canal (Fig. 22). The infraorbital line is continued on to the body as the dorsal line of free organs (Figs. 22 and 24).

2. *Supraorbital line.* — There are seven organs in this line. The first, a free organ, is situated near the median line, a little anterior to the opening of the posterior nares (Pl. XX, Fig. 22). Organs 2–6 are enclosed in a canal, while the seventh is a free organ occupying a position apparently outside the line and on the top of the head (Pl. XVII, Fig. 2 ; Pl. XX, Fig. 22). There is evidence of the presence of the supra-temporal cross-commissure, although the canal seen in Amia is wanting in Batrachus. In one specimen, 12 cm. in length, the line was conspicuous, as two extra organs were present in this region of the head. In Pl. XX, Fig. 22, *st.com.*, the position of the line is indicated. The middle pit line of Amia may be represented in Batrachus by the organ just dorsal to the temporal canal (Fig. 22, *m.l.*).

Four organs on the top of the head, extending on to the trunk each side of the first dorsal fin, constitute what is designated by Allis as the dorsal body line in Amia. (Compare Figs. 21–23.)

3. *Operculo-mandibular line.* — The first organ of this line is found on the lower side at the symphysis of the mandible. There is no commissural connection here between the two sides of the head. Four organs, which never become enclosed in a canal (Fig. 3), occupy a depression which appears as an

open groove in the bone (Fig. 5). The succeeding organs, 5–7, are within a canal in the articular bone (Fig. 5). At the angle of the jaw the opercular division begins, and consists of four enclosed organs (8–11) with one (12) free organ near the temporal region (Fig. 22). Outside of these twelve organs of the operculo-mandibular line there are accessory lines of free organs. On the mandible there is a short line of three organs (Pl. XVII, Fig. 3, *ac.md.l.*) anterior and parallel to the canal. Near the pore at the junction of the two portions of the operculo-mandibular line there are two free organs (*mdl.*) (Figs. 1, 3, and 22), while on the operculum two lines of free organs diverge at right angles to the canal in the preoperculum, the more dorsal (*d.o.l.*) having four, and the other (*v.o.l.*) three organs (Fig. 22).

4. *Body lines.* — There are three lines of free organs on the side of the body (Fig. 24); the most dorsal, of twenty-seven organs, being a continuation of the infraorbital, the middle line appearing as a branch from this line, represented by only a few scattered organs, usually eleven, and the ventral line, of twenty-seven organs, extending from a point in front of the ventral along the border of the anal fin. Continuations of these lines are found on the caudal fin, but the organs are somewhat diminished in size toward the posterior end of the body. The usual number on the caudal fin is four.

## *Canals.*

The canals enclosing lateral line organs are found only on the head, and these present a rudimentary, perhaps vestigial condition in Batrachus.

From Fig. 22 it appears that the infraorbital line throughout its extent has only two short canals, one on the maxillary branch containing two organs, and the other in the temporal region enclosing only one. The supraorbital line, on the other hand, exhibits the opposite condition, in that all the organs of the line are enclosed in a canal with the exception of a free organ at each end of the line. The operculo-mandibular canal is well developed, only five of the twelve organs being super-

ficial. There is a direct union of the canals of the two sides of the head between the eyes, but no organ is developed in this commissural portion (Fig. 23; also Fig. 4).

### *Pores.*

For a complete understanding of the relation of the pores to the canals, a knowledge of their mode of development is necessary. Each organ becomes enclosed in a short canal (Cut 1), the two openings of which are called by Allis terminal or half pores. By the union of these half pores, the so-called primary pores of the young Amia are formed.

In the case of Amia there is a subsequent process of division of these primary pores, resulting in the dendritic systems of the adult fish. The pores in Batrachus correspond to the terminal and primary pores of Allis, as shown in the diagram


Cut 1. — Diagrammatic representation of the formation of a primary pore: *a*, *b*, and *c*, two terminal pores approaching each other and fusing; *d*, primary pore.

representing the post-larval stage of Amia. (Compare Figs. 21 and 22.) In the supraorbital line of Batrachus the *primary pores have become fused*, so that only the two terminal pores are present, and no pore marks the union of the canals between the eyes, as seen in Cottus gobio. These pores, in process of fusion, may be observed during the development of the canals in the young fish.

It seems all-important that the term *pore* be restricted in its application to the *openings into the canals.* In consequence of the indiscriminate use of this word, it is often difficult to understand the statements of some writers. A puzzling case is presented in a description of the canals of Polyodon by a writer (6) on the Sensory System of Ganoids, where *the "cluster pores" are described as openings of canals, and figured as sense organs!*

A recent writer (7) in alluding to this subject says: "The word *pore* is inappropriate in Amphibia if used in the same

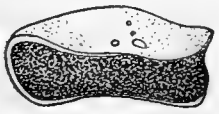
sense as in fishes," as may be easily understood when it is known that *no canals exist in the Amphibia.*

### *Relation of Canals to Cranial Bones.*

From an examination of the skull (Fig. 4) it appears that grooves or open channels in the bones serve as protection for the organs. In Batrachus the only cranial bones which become modified to give protection to the lateral line organs are the frontal, dentary, and articular bones, the preoperculum, and an accessory membrane bone in the maxillary branch of the infraorbital. The curious T-shaped arrangement of the upper surface of the frontal bones where the canals of the two sides of the head unite, has given the specific name (tau) to the species under consideration. These channels are spaces between ridges of bone projecting from the surface and partially surrounding the membranous tube containing the sense organs. They vary in diameter in the different regions of the head. In the opercular region this membranous tube occupies the space (Fig. 4) between the outer edges of the two lamellae of bone forming the preopercle. In the canal of the maxillary branch the accessory membrane bone appears as though folded together to enclose the canal (Fig. 4, *ac.b.*). In the mandible there is the nearest possible approach to a closed bony canal (Fig. 5), while in the case of the temporal canal there is no cranial bone involved. This short canal lies outside the muscles which cover the squamosal and occipital bones, and consists of a tough fibrous or semi-cartilaginous covering within which is the lining epithelial layer (Fig. 22, T.C.). Leydig (8) figures a similar formation in Chimaera. The supporting substance is described as consisting of incomplete rings, one behind the other, comparable to the rings of the trachea, and the free ends of these rings are represented as branching. In cross-sections of the temporal canal in Batrachus a very similar structure is seen.

At the anterior end of the supraorbital canal there is a scale-like cartilaginous formation, by means of which the canal is extended across between the two openings (Fig. 22) of the

nasal tube. This scale bears some resemblance to the cartilaginous tube of the temporal canal, yet is unlike it, and seems to be a peculiar structure found in no other part of the canal system of Batrachus. Something very similar is found in the canal of the trunk in Cottus gobio (Cut 2), as described and figured by Bodenstein (9).



Cut 2.—Scale from trunk canal of Cottus gobio.

The nasal tube itself is a canal belonging to this system which never becomes surrounded by any bony formation. In this connection it may be stated that there is good reason for regarding the semicircular canals of the ear as belonging to the lateral line system, although shut off entirely from the surface of the body. This view has been advocated by Ayers (10) and other writers.

### *Number and Position of Organs.*

In Batrachus the organs in canals are identical with the so-called free organs, the only difference being the fact that the free organs, being situated on dermal papillae, have a slightly different form.

The number of organs on the head is 128, and on the body 140, making a total of 268 organs on the entire surface of the head and body. The number enclosed in canals is only 30, making the number of free organs 238. There is no indication that the number of organs increases by multiplication during the life of the fish, and the "nerve ridges" described by Allis (2) have never been found in Batrachus. The "pit organs" of Amia, assigned to the same general class of nerve hillocks, are yet said to differ greatly from the canal organs in "shape, arrangement, and methods of multiplication." From the description, however, there seems little evidence of greater difference than between the enclosed and free organs of Batrachus, except, possibly, in the size. It seems quite impossible to arrange them in two separate groups in the case of Batrachus, as they replace so constantly the regular canal organs. The enclosure of organs within a canal seems quite incidental and secondary. The absence of accessory lines of pit organs is

quite noticeable in Batrachus, as also the numerous "surface sense organs" (terminal buds) described by Allis (2) on the head of Amia.

### *Variations.*

Frequent variations in the number and position of the organs have been noted. There may be five, six, or seven organs in the antorbital portion of the infraorbital line. The number in the suborbital may be eight or nine. In the mandibular line at the place of union of the opercular and mandibular divisions one organ is often wanting.

Two extra organs — one on each side of the head — occurred in the case of one specimen, confirming the opinion that the free organs of this region are homologous with those of the commissural canal in the occipital region of Amia. (Compare Figs. 21 and 22.)

On the body the variation is still more marked, the two sides seldom having exactly the same number or arrangement of organs.

On one large specimen there was the following arrangement:

In the dorsal body line of the right side, 25, left 29.
" " middle " " " " " " 11, " 8.
" " ventral " " " " " " 26, " 27.

At the anterior end of the ventral line in another specimen one organ was lacking on each side. The number may be four, five, or six on the caudal fin.

### *Innervation.*

The method most successfully employed for determining the course of the nerves was maceration of the adult fish in nitric acid. After being kept for some time in a weak solution, not only the large nerve trunks could be easily followed, but the bundles composing these trunks could be separated, the connective tissue sheath having been dissolved. It thus became possible to demonstrate the course of the different components of nerves enclosed in the same sheath.

By reference to the diagrams (Figs. 22 and 23) which represent the side and dorsal view of the head and anterior part of the body of an adult toadfish, the course of the nerves may be traced after their exit from the skull. Fig. 21 is reproduced from Allis's plate for purposes of comparison, as it is of interest to note the general resemblances and slight differences which appear in comparing the teleost Batrachus with the ganoid Amia. As may be observed, the number of organs in the different lines and their mode of innervation correspond in a surprising manner.

The lateral line system in the head of Batrachus is innervated by dorsal branches of the VII and X cranial nerves.

### *The VII Nerve.*

*The supraorbital line* is innervated by the R. ophthalmicus superfacialis facialis (Fig. 22). This branch arises from the ganglion lying above the Gasserian ganglion (Fig. 13), and runs along the inner margin of the orbit in close association with the ophthalmic branch of the trigeminus. There is an evident interchange of fibres in one place, and the two nerves are included in the same sheath for a short distance near their peripheral termination. Each organ is supplied by a short branch, which enters the bony canal immediately below the organ. Organ No. 7 being a free organ, and on the top of the head, yet belongs to this supraorbital line of organs, as may be seen by tracing its development and its innervation. As Allis has shown in Amia, the supraorbital line is widely separated, at an early period of development, from the infraorbital, at the point where later there is a union of the two canals.

*Infraorbital line.* — The organs in the pre-auditory part of the infraorbital line are innervated by the R. buccalis facialis (Figs. 22 and 23, *buc.f.*). This branch arises from the facial (Fig. 13), lying above the Gasserian ganglion, and immediately divides, sending a comparatively small number of fibres (Fig. 13, *buc.f.*$^2$), to the outer angle of the orbit to supply the eight organs of the suborbital portion of the infraorbital. The

main portion of the buccalis passes directly downward to the floor of the orbit (Fig. 13, *buc.f.*[1]) enclosed for some distance in the same sheath with the maxillaris of the fifth. It then again divides, one branch being directed toward the median line supplying the six antorbital organs, while the other sends branches to the seven organs which constitute the maxillary portion of the infraorbital line (Fig. 23). The two organs (9, 10) of the infraorbital line, corresponding to those innervated by the otic branch in Amia (Fig. 21), are in Batrachus supplied by a branch from the R. buccalis facialis (Figs. 22 and 23).

*Operculo-mandibular line.* — The organs of this line are innervated by the R. mandibularis externus facialis. Organ 12, — a free organ, — together with the most dorsal of the two branch lines of free organs on the operculum (Fig. 22, *d.o.l.*), are innervated by a branch of the hyoideo-mandibularis facialis, before the externus has separated from it. It leaves the main trunk through the foramen at the base of the opercular spine.

Organs 8–11 are supplied by branches which pass from the externus between the bony lamellae of the preoperculum to the canal occupying the space between the outer edges of these lamellae. Between organs 9 and 10 a branch is given off to the three free organs forming the ventral line on the operculum (Fig. 22, *v.o.l.*).

There are two free organs situated near the large pore which marks the union of the opercular and mandibular portions of the line, and which seem to correspond to the mandibular pit line of Amia (Fig. 21, *m.d.l.*), which are innervated by branches from the externus. In the same way the two free organs at the angle of the mouth in Batrachus may easily be identified with the vertical cheek line in Amia (Fig. 21, *c.l.*), also supplied by a branch from the externus.

The three canal organs of the mandible are innervated by the externus, as also the four organs in the groove at the anterior part of the mandible (Fig. 3), while the three superficial organs in a line parallel with them are also supplied by a branch of this same nerve (Fig. 22, *ac.md.l.*).

There seems to be an interesting peculiarity in the innervation of the body lines of Batrachus. The N. lineae lateralis does not supply the line of sense organs continuous with the infraorbital of the head, but it does send branches to some of the scattered organs of the middle line. The dorsal and ventral lines of the body are innervated, in part at least, by the R. recurrens facialis. This nerve emerging from the *ventral* branch of the dorsal VII (Fig. 13) turns directly backward within the cranial cavity; it passes obliquely through the cranial wall and through a loop in the glosso-pharyngeal, beyond which it forms an anastomosis with an ascending branch from the posterior root of the vagus, at a point just behind the auditory capsule. The position of the R. recurrens facialis is superficial to the N. lineae lateralis, and it extends on to the body just underneath the skin. It divides immediately (Fig. 22), sending one branch toward the dorsal region supplying the organs of the anterior portion of the dorsal line, while the ventral branch curves around behind the base of the pectoral fin innervating the anterior organs of the ventral line.

*The X or vagus nerve.* — The anterior root of the vagus nerve arising from the dorsal region of the medulla (Fig. 12, *X an.r.*), does not possess any distinct ganglion. It runs backward and outward, crossing the main root of the vagus, with which it is connected by a few fibres, and after leaving the cranium by the foramen in the occipital is continued on the body as the N. lineae lateralis. It courses deeply underneath the muscles for some distance, becoming superficial at the posterior portion of the body.

Although this is the main lateral line nerve, *it seems to innervate only a few of the organs on the body of Batrachus.*

The supratemporal branch of the vagus is composed mainly of anterior root fibres (Fig. 13, *st.v.*[1]). It arises intracranially, passing upward and leaving the skull through a foramen in the supraoccipital (Fig. 4, *o.c.f.*). It then turns forward, supplying the canal organ of the temporal region (Figs. 22 and 23) and three other organs on the top of the head. The most anterior of the three organs may be considered as representing the middle dorsal line of pit organs, which in Amia are innervated

by branches of the IX, while the two others are probably homologous with the organs forming the cross-commissure of Amia. (Compare Figs. 21 and 23.)

Another branch (*v.*²), arising from the anterior root of the vagus just outside the cranium, and taking a course upward and backward, innervates the four organs of the dorsal line (Fig. 22, *dl.*).

Fig. 13 shows an intracranial commissure between the VII and X. The branch from X which anastomoses with the R. recurrens facialis (Fig. 13) arises from the *posterior root* of the vagus. It is also evident that the fibres of the R. recurrens facialis emerge with the *ventral root fibres* of the VII. It is probable that the components of this nerve have a different central termination in the medulla from the dorsal branches of the VII and the anterior root of the X. The innervation of the body lines in Batrachus presents a somewhat puzzling problem, which can only be solved by a careful study of the terminations or origin of the fibres in the medulla. The *apparent* course of the nerves is often deceptive, as fibres having *different central connections* are enclosed in the *same sheath* outside of the medulla, or, the central connections being the same, the fibres follow different pathways to their destination. A case in point is that of the glosso-pharyngeal, which seems to take no part in the innervation of sense organs in Batrachus, although in Amia one canal organ and a line of pit organs are supplied by that nerve.[1] It is probable that the fibres innervating the organs are enclosed sometimes with the IX and sometimes with the X nerve.

The attempt to homologize the body lines of Batrachus seems useless until a better knowledge of the components of the so-called R. recurrens facialis is obtained. It seems probable that this nerve is identical with the R. dorsalis recurrens trigemini (Stannius), which is said to innervate a line of end buds at the base of the dorsal fin in Siluroids, but the dorsal body line of organs in Batrachus would hardly seem homologous with this line of end buds.

[1] In a recent paper (*Journal of Morphology*, vol. xii, p. 747) Allis has shown that the "so-called dorsal root" of the IX is composed of fibres from the lateral line root of X.

### *General Considerations.*

If one may judge from the contributions to this subject by various investigators, it is becoming evident that the lateral line system may take rank among the organs of special sense.

The connection of the olfactory, optic, and auditory organs with the central nervous system is effected by means of special pairs of cranial nerves originating in definite centers within the brain. On the other hand, the sensations of touch are mediated by cutaneous nerves which seem to be so universally distributed as to suggest the idea that the skin itself may be regarded as an immense sense organ and its innervation correspondingly general.

The system of the lateral line has usually been regarded as composed of organs of the more *generalized* type. Their wide distribution over the entire head and body would favor this conclusion, but the study of the cranial nerves of Amphibia brings into view several significant facts. In his recent paper, Strong (11) calls attention to a "most beautiful extirpation experiment in nature." The tadpole has the sense organs found in fishes and the Urodeles, and these organs are innervated by certain dorsal branches of the cranial nerves. When the tadpole becomes a frog, and these organs disappear from the skin, the dorsal branches supplying them become atrophied. As regards the innervation of the lateral line organs, there seems to be a remarkable agreement between the Urodela, larval forms of Anura, and the fishes. In general, the arrangement seems to be the same, inasmuch as dorsal branches of the VII and X cranial nerves supply these organs. This has been shown in the case of amphibians (11), selachians (12), two ganoids and one dipnoid (13), but among teleosts the matter has been in doubt. Batrachus is certainly one teleost in which the dorsal branches are present and innervate the lateral line organs.

In his analysis of the cranial nerves of Amphibia, Strong gives a description of the different nerve components distinguishable by the nature of their fibres, peripheral distribution, and internal origin:

He describes a *general* cutaneous component and a *special* cutaneous or lateral line component, the dorsal branches of which innervate the organs of the lateral line. These branches are coarse fibered and therefore distinguishable in sections, while their internal origin or termination is the tuberculum acusticum, a portion of the medulla which is greatly developed in fishes. If the lateral line component has its origin in the tuberculum acusticum, we have good reason to conclude that the localization of function in the medulla is as definite for these widely distributed organs as it is for the more circumscribed patches of sensory epithelium seen in the case of the ear, eye, or olfactory organ.

The ear furnishes a fine illustration of this subject, and seems like a connecting link between the system of lateral line organs from which it has probably originated, and the most highly modified sensory structure in Vertebrates — the eye. Ayers (10) has shown that the auditory organ is in reality a series of canal organs innervated by two distinct cranial nerves which he regards as possibly dorsal roots of VII and IX.

## II. LARVAL FORMS.

### *Origin of the Lateral Line System.*

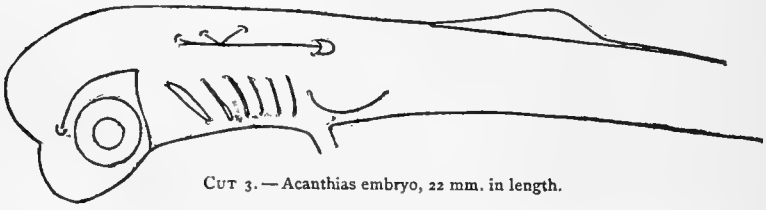
1. *Lateral line sense organs.*—Sections of early stages in the development of Batrachus show thickenings of the ectoderm in the region behind the eyes. In sections of later stages these areas of thickened ectoderm have become invaginated to form the auditory vesicles. Immediately after the closure of the auditory pits, thickenings of the lower layer of the ectoderm are observed on each side of the head in the pre-auditory region. From this thickened area two cords extend, one above and another below the eye. These cords are the rudiments of the supraorbital and infraorbital lines of sense organs.

Simultaneously in the post-auditory region there appear similar thickenings of the ectoderm which extend rapidly backward on to the trunk. In very young embryos this line advances along the side of the body with an enlargement at the growing end.

These thickenings of the ectoderm are described by H. V. Wilson (14) as "sensory tracts," and he maintains that in the bass, unlike what has been observed in selachians, in Amia and in the trout, the lateral line originates in the form of "sensory sacs," which later on become flattened out into the "patches" described by other authors. There is no dissent from the view that the auditory region is the place where the lateral line system originates, but the occurrence of these "sensory sacs" appears to be peculiar to the bass. Wilson states that the ear, branchial organ, and the first of a series of organs extending on to the body, are derived from this "common sensory furrow." In Batrachus there is no definite furrow present, and the "branchial sense organ" described as "functional in the later stages of embryonic and during larval life" is certainly not "histologically differentiated" as in the bass. There is no sign of an organ composed of "sense cells with short stiff hairs," as described by Wilson in an embryo of fifty-nine hours.

Fig. 17 shows the growing end of this line as seen in a preparation of the skin of an embryo 5 mm. in length. After fixing in picro-sulphuric acid and slight maceration in water, if the skin is removed, stained in alum cochineal, and mounted in glycerine, the proliferating cells in the lower layer of the ectoderm may be clearly seen. A horizontal section of this is shown in Fig. 18, at the time when the growing point has reached only a short distance behind the pectoral fin. A more highly magnified view of a portion of the same is seen in Fig. 19. A comparison of the lateral line of Batrachus at this stage with the same structure in an Acanthias embryo is of interest (Fig. 14). In Acanthias the lateral line is quite conspicuous. In an embryo of 22 mm. (Cut 3) it is easily seen with a hand lens, as a prominent, somewhat flattened ridge, extending backward above the branchial region and along the sides of the body. There is a curious fold of the epidermis, the so-called "pocket," which covers the growing end of the line. Pl. XIX, Fig. 14, shows a horizontal section of an embryo 17 mm. in length, from which it is evident that the "pocket" consists of a reduplicature of the skin accompanying the enlarged growing point.

The branches of this system on the head also show this peculiar fold (Cut 3), the significance of which it is hard to discover. In connection with this fold, Mitrophanow (15) describes the formation of canals, but gives no figures that illustrate the manner of their formation. Behind the dorsal fin, as seen in older embryos, the "pocket" becomes greatly elongated, and suggests the existence of a canal in that region, but furnishes no clew to the condition that is supposed to exist in the anterior part of the body of the adult Acanthias. Cut 4 is a cross-section of the anterior part of the line at this stage.



CUT 3. — Acanthias embryo, 22 mm. in length.

This subject has not been sufficiently investigated to afford a satisfactory basis for comparisons. Beard (16) mentions this growing end of the line as "plowing its way backward through the indifferent ectoderm." The appearance of the structure in Acanthias would suggest this idea.

Balfour (17) describes the lateral line of Syllium as appearing "in the form of a linear thickening of the inner row of cells of the external epiblast on each side, at the level of the notochord." He says that at this time it shows no segmental character, and he also notes the interesting fact of the "broadening at the growing point." He probably has reference to this remarkable fold of the epiblast when he speaks of the "perfectly abrupt" termination of the line. He also mentions the contrast between the narrow anterior and the broad terminal portion of the line. This thinning out of the anterior portion of the lateral line is noticeable in Batrachus. Allis describes and gives figures of surface preparations showing the same appearance of the line in Amia. "The ends of these lines are enlarged, that of the lateral line sometimes forming a large and prominent swelling."

Hoffmann (18) regards the sense organs as arising segmentally, and gives no account of the growth of the rudiment

of sense organs on the side of the body. H. V. Wilson (15) has evidently found the line only in the form of a slender cord on the posterior part of the body, and makes no mention of any enlargement at the growing end. From figures in a recent paper on Necturus, by Miss Platt (19), this enlarged growing point is shown as quite conspicuous. In selachians, ganoids, and amphibia we have evidence of this mode of growth of the sense organ rudiment, but no figures or descriptions of the enlarged growing point of the lateral line of any teleost have been published, so far as I am aware.

Fig. 6 represents the condition of a Batrachus embryo about the time of hatching and when the embryo is still attached to the yolk sac. The principal organs of the different lines can now be distinguished in surface preparations, but a more satisfactory showing of the exact number and position of the organs, as well as of the connecting strand, can be obtained from preparations of the skin, as previously described.

Regarding the canal and free organs as identical, the development may be briefly outlined as follows: In Batrachus, as in Amia, the growing line of sensory epithelium begins to present the appearance indicated in Fig. 15, which is a camera drawing from a preparation of the skin at a stage somewhat earlier than that shown in Fig. 6. The cells destined to form the sensory portion of the organ begin to elongate and arrange themselves in a definite manner, suggesting the name "hillock" given by Merkel (20) to this class of organs. At the summit there soon appears a clear vacuolar space toward which the upper portion of the cells is directed. The "hillock" formed in the lower layer of the skin soon pushes its way to the surface and gradually takes on the characteristics of an adult organ. This process has been fully described by Allis (2).

The sensory and supporting cells are very much alike in the organs of Batrachus, although the cells in the center of the "hillock" are pear-shaped and somewhat shorter than those of the peripheral part of the organ. From Fig. 16, which is a section of a side organ of a fish of one year old, the shape of the adult organ is evidently that of a cone hollowed at the base.

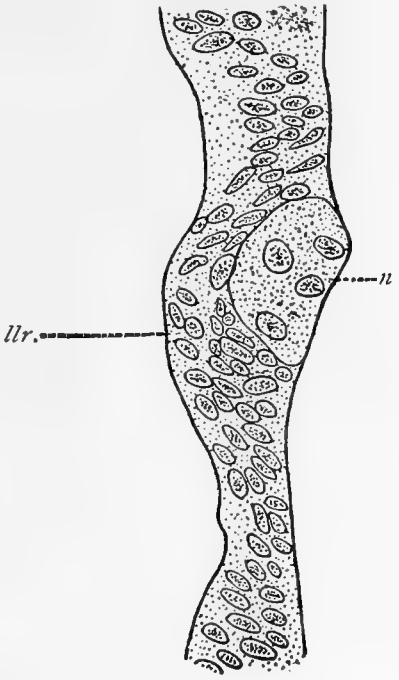
Soon after the organ has reached the surface, there appears on the summit a structure known as the "hyaline tube" or "cupola." This was seen on specimens 2 cm. in length, being easily observed with low magnifying power on the living fish, some chloroform being added to the water to quiet the fish during the observation. This tube measured .10 mm. in length and .01 in breadth. From sections through the canal organs of older fish, it is apparent that this "hyaline tube" is present after the enclosure of the organs in canals. There is little doubt in regard to the nature of this hyaline structure. The cells of the organ, in common with other epidermal cells, may secrete a cuticular substance on the free ends of the cells. In the case of the hair cells this secretion is in the form of hairs or bristles. These hairs may coalesce, forming a continuous membrane, surrounding the central portion of the hillock, thus forming the so-called tube, which is frequently present. The hairs of the most central cells may remain separate within this tube, as Leydig (1) observes in his most recent paper on this subject.

The "terminal buds" of Merkel or "end buds" of other writers are not found on the surface of the head or trunk of Batrachus, but they occur in the mouth and branchial cavities. These organs are much smaller than the hillocks on the surface of the body, and very little is known in regard to their innervation.

2. *Lateral line nerves.* — In his "Elasmobranch Fishes" Balfour (17) says that, in considering the subject of the lateral line system, we are dealing with two distinct structures, (1) the modified epidermis seen in certain lines along the sides on the head and body, and (2) the accompanying nerves.

The origin of the organs from the modified ectodermal cells has been demonstrated, but the mode of origin of the accompanying nerves is not so well understood. Balfour (17), following what he believed to be the analogy of cranial nerves in general, held that the dorsal branches which supply the sense organs grow out from the brain to these organs. On the other hand, Götte (21), Semper (22), van Wijhe (23), and Beard (16) consider it certain that the cells from the ectoderm contribute

to the formation of these branches. According to Hoffmann (18), the lateral nerve in Salmo arises from a string of cells in the nervous layer of the ectoderm some time previous to the development of the organs. This string gradually moves out of the ectoderm, coming to lie at some distance internal to it, but connected at intervals by short side branchlets with the locality where the future segmental sense organs are to arise. Hoffmann's (18) observations were made on embryos of a teleost, which he regards as a less favorable form than the selachian, in which, according to Semper (22), it is uncommonly easy to show the origin of the lateral nerve.




Cut 4. — Cross-section of lateral line of Acanthias embryo.

In the section of the Acanthias embryo (Fig. 14), there is an evident extension of nerve fibres from the vagus ganglion accompanying the lateral line rudiment. In cross-section these fibres are seen to constitute at this stage a part of the lateral line (Cut 4). It will be necessary to study the changes taking place in the later stages before a final conclusion can be drawn, but it would appear that the thickened ectoderm forming the lateral line and the extension of the outgrowths of the ganglion cells were associated during the early history of the structure.

Hoffmann's (18) description of the origin of the nerve becomes more intelligible after the study of the selachian embryo, although in both the case of Salmo and Acanthias the *exact mode of origin of the sense organs remains uncertain.* In Batrachus, on the other hand, the origin of the sense organs

is easily demonstrated, while the *origin of the nerves and their connection with the organs becomes the great problem, as in the case of other teleosts.*

In stages represented in Fig. 18 it is impossible to detect any *nerve fibres* accompanying the growing line on the side of the body. The whole line has the appearance of being an *extension of the mass of ganglion cells.* This seems the more striking as the entire string of cells constituting the rudiment of the lateral line in Batrachus seems to occupy the same relative position as the extension of *ganglionic fibres* in Acanthias.

Wilson (14) states that he has been unable to trace the origin of nerves in the Bass. He says in regard to the lateral branch of the vagus, that he could not distinguish it "during embryonic life" nor "in the larvae of two or three days."

It is difficult to reconcile Hoffmann's (18) observations on Salmo with the facts brought out by Wilson (14) or with the conditions existing in Batrachus. That the origin of the sense organ rudiment precedes the appearance of the nerve in both the Bass and Batrachus can hardly be doubted, while from the description of the "growing sensory tissue" in the skin of Amia, Allis (2) surely conveys the idea of the early appearance of the sense organ rudiment.

3. *Formation of canals.* — Figs. 7–11 represent the appearance of the head of Batrachus during the period of the enclosure of the organs in canals. The plates of Allis show in detail the different steps of this process of canal formation, and a very full account of the same is to be found in the admirable paper on the development of the lateral line organs of Amia. Previous to this paper, we have the accounts of canal formation in Cottus gobio by Bodenstein (9), and in Platería by Schulze (24) and Solger (25), but the illustrations of the subject, as well as the accounts, are not so full and clear as in the case of Amia.

The appearance of the head during these stages is almost identical in Amia and Batrachus. At the time of hatching, the organs are not apparent on the surface, but after treatment with picro-sulphuric acid they may be seen below the surface as whitish spots or irregular lines (Fig. 6).

The canals are formed in sections, as described by Allis (2): "After a developing canal organ has reached the surface, it begins to sink, carrying with it the surrounding tissues, thus forming a small pit at the bottom of which the organ lies. Lips grow upward and inward from the edges of the pit, and meeting above the organ, form a short canal, the openings of which are inclined to the general surface and give to the canal a tunnel-like appearance." In Figs. 7 and 8 the organs have begun to sink below the level of the surface and form linear areas of depression.

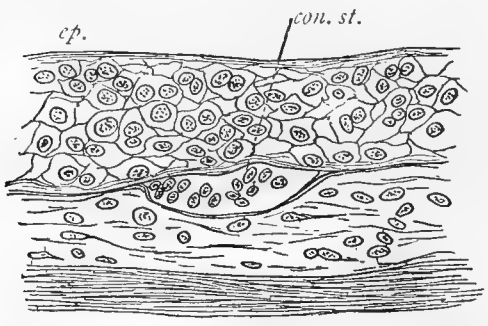
In Figs. 9, 10, and 11 the process has been continued and the organs are partially enclosed by the approaching lips of the canal, but complete fusion has not taken place. This condition is permanent in some forms, as Chimaera and Polyodon, open grooves taking the place of canals in the adult. The process of enclosure goes on unequally; the most anterior organs are the first to become enclosed. In Fig. 10 the line of fusion of the nasal tube is distinctly seen, and the two half pores which are formed constitute the anterior and posterior nares. In the supraorbital line the process of fusion is carried out most completely, the short canals coalescing and therefore no primary pores formed, the terminal or half pores only being present (Figs. 2 and 23). A comparison of the commissural canal between the eyes, so prominent at this stage (Fig. 10), with the bony channels on the frontal bones (Fig. 4) is instructive, as showing the effect of the flattening of the head and the closer approximation relatively of the eyes in the adult.

In the case of the operculo-mandibular line (Fig. 9) the opercular portion is seen to form independently of the mandibular division, and the double or primary pore which marks their union remains larger than the others of the line (Fig. 22). In the mandibular portion of the line the four anterior organs are never enclosed in a canal, but retain the open groove condition in the adult (Fig. 3).

4. *Connecting strand.* — While examining adult specimens of Batrachus which were partially macerated in nitric acid my attention was attracted by a very well-defined strand connecting the organs on the side of the body. This structure had the

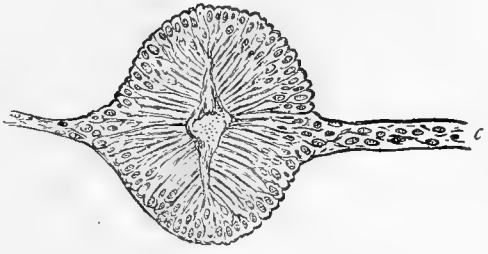
appearance of the commissures connecting the ganglia of the sympathetic system, and from the fact that it resisted the action of nitric acid I inferred that it was nerve tissue. In direct sunlight, by aid of an ordinary lens, this cord was plainly to be seen, and its connection at either end with the cells near the summit of the sense organs was evident.

The appearance of this structure in connection with a free organ is shown in a section from the side of the body of a specimen 10 cm. in length (Fig. 16, *con.st.*). As may be seen, the strand near the organ has a diameter greater than that of the nerve supplying the organ, and it extends in a sort of festoon from the summit of one organ to that of the next in the line, becoming narrower midway between the organs. It extends below the skin into the thick felt-like layers of connective tissue occupying the space between the skin and the muscles. This cord is also found connecting the organs of the supra- and infraorbital lines in the head, as well as those of the operculo-mandibular line, and even where free organs seem quite disconnected, as in case of organs on the top of the head, there is at least a short extension of this cord on each side of the organ. In Figs. 22 and 23, the strand is represented in blue. A cord of cells is found on the floor in the epithelial lining of the canals (Figs. 22 and 23). It therefore becomes evident that in the case of Batrachus tau the connecting strand constitutes a prominent feature throughout the lateral line system in the adult fish (Cut 5). Bodenstein (9) describes this structure in the adult Cottus gobio, and says that the strand extends from the *center* of one organ to the next. This is hardly the case in Batrachus (Fig. 16), as the cord evidently terminates at the *summit* of the organ, among the supporting



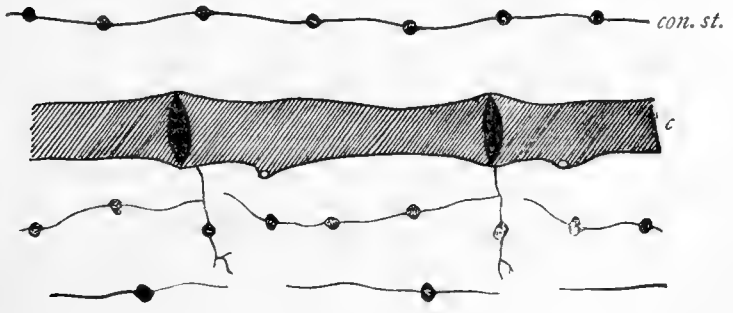

CUT 5.—A cross-section of temporal canal of Batrachus, showing the strand in floor of canal.

cells, having no connection with the nerve, as suggested by Bodenstein, when he speaks of the possibility of its forming anastomoses with the nerves in the series of sensory hillocks. Emery (26) describes what he calls "epithelial canals" in the adult Fierasfer, and his figures leave no doubt as to their homology with the connecting strand of Batrachus (Cuts 6 and 7). No mention is made of any connecting canals between the *canal organs*, but they are evidently well developed between the "nerve buttons" (pit organs) of the accessory lines.




Cut 6. — Copied from Fig. 58, Emery (26).

The fact that these "canals" sometimes branch and end blindly (Cut 7) is a peculiar characteristic if these canals are functional. Exactly similar peculiarities are noticed in the case of the strand in Batrachus. The free organs situated in a line parallel with the canal on the mandible have the strand

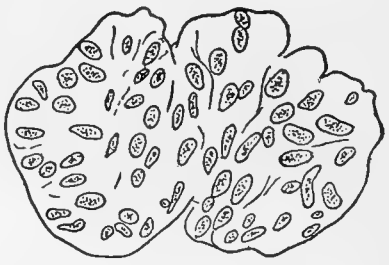



Cut 7. — Copied from Fig. 6, Emery (26).

directed at right angles to the canal, and in one case the end of the cord was branched in a similar way to that figured by Emery. In a series of cross-sections the irregular outline of this strand in Batrachus becomes evident (Cut 8). There is some indication of its being fibrous in structure, and

often near the organ a suggestion of a lumen is noticed, especially in longitudinal sections.

According to Leydig (1), Feé (27) seems to have figured this connecting strand, but makes no allusion to it. Solger (25) refers to the "side organ chains," in the case of Acerina cernua and Lota vulgaris, and speaks of the chain as consisting of "marrowless nerve fibres enclosed in a nucleated sheath." Merkel (20) speaks of "modified (cutis) epithelium" and suggests that the connecting strand may be the vestige of a *canal!* The presence, however, of both the canal and the connecting strand, one found within the other, as in Batrachus, would overthrow any such supposition. Carrière (28) thinks there is no possibility that this "chain" is composed of nerve fibres. Ryder (29) speaks of "faint filamentous prolongations" from the organs. In a figure of Savi's (40) vesicles there is a connecting cord shown and described as "filament anastomotique," which suggests the same structure.



CUT 8. — Cross-section of strand between organs 9 and 10 of infraorbital line of Batrachus.

Leydig (1) has examined this peculiar structure in Gobio, Rhodeus, Salmo, and Anguilla, and although reaching no conclusion as to its significance, says that the strand does not consist of nerve elements, but principally of epithelial cells which enclose a space that may be considered a lymph passage, or, in some cases, no lumen being present, the strand presents a fibrous or ligamentous appearance. He regards the "epithelial canals" of Fierasfer as lymph channels. Leydig (1) further observes that although he has not seen the epithelial thickenings out of which the sense hillocks arise, it is probable that the strand is derived from these thickenings. From this point of view the strand would be a remnant of an epithelial growth which separates from the epidermis and forms the foundation of the sense hillocks. Leydig (1) utterly discards the idea that this structure is in any way connected with the later forming canal.

In regard to the origin of the strand, my observations on the embryos and larval forms of Batrachus would tend to confirm the opinion expressed by Leydig. Whatever the function may be, *its origin from the sense organ rudiment is not to be doubted.* In very young embryos the growth of the sensory tissue is easily demonstrated, as shown elsewhere. In the larval fish 15 mm. long, just after the yolk has become absorbed, the strand is distinctly seen in preparation of the skin, the cells of the strand between the organs still retaining much the same appearance as in earlier stages (Fig. 15, *con.st.*). Later, however, the cells undergo a change so that the tissue appears as seen in Fig. 16, *con.st.*

### *Comparison with Other Teleosts.*

1. *Lophius piscatorius.* — The goosefish resembles the toadfish in being destitute of scales and in having similar tentacular appendages in various parts of the body (Guitel, 30). The sense organs are not enclosed in canals, but are protected by projections of the skin, as in the case of the free organs of Batrachus. The maxillary portion of the infraorbital line of organs is greatly developed and the suborbital is wanting. The innervation is quite similar in the two forms, the dorsal branches of the VII being quite distinct from the V.

2. *Cottus gobio.* — Bodenstein (9) has described the "connecting strand" in the adult Cottus and represents it in his figures as on the floor of the canals. From his description of the skin and the appearance of the canal organs, there is a striking similarity between the two forms.

3. *Amiurus.* — Batrachus and this common fresh-water form have several characteristics in common. The naked skin, closely studded with gigantic gland cells, the depressed head, and general shape of the body is the same, but the sense organs of the trunk in Amiurus are, for the most part, in canals. The interesting comparison is in respect to the course of the R. dorsalis recurrens facialis, which has been wrongly called "*trigemini.*" In Amiurus, according to Wright (31) and Pollard (32), this arises from a "posterior dorsally placed gangli-

onic extension and passes upwards intracranially to the parietal bone," and from thence on to the body innervating a dorsal line of sense organs. This nerve is undoubtedly homologous with the R. recurrens of Batrachus, although taking a somewhat different course. It receives a branch from the vagus, and in this respect resembles the R. recurrens facialis of Batrachus.

4. *In Fierasfer* the "connecting strand" is well developed, and although Emery (26) describes this structure as an "epithelial *canal*," still the evidence is hardly conclusive from his figures.

5. *Ganoids.* — As already shown, Batrachus and Amia have many of the same characteristics, but in Batrachus the canals are never entirely enclosed within the bones of the skull, nor is the elaborate system of branching canals with their numerous groups of pores to be found. Allis (2) has shown that the trigeminus takes no part in the innervation of the canal organs of Amia. The terminal buds found in such abundance on the surface of the head of Amia are not present in Batrachus.

6. *Selachians.* — The comparison between the lateral line of Acanthias and Batrachus, which has already been made, shows the differences that will probably be found to exist in the mode of origin of this system in the two groups of fishes. So far as the innervation is concerned, there is great similarity between Batrachus and selachians. Ewart (12) has shown that the lateral line organs are supplied by the dorsal branches of the VII and X cranial nerves.

7. *Dipnoids.* — Pinkus (13) has shown that the commissure connecting the VII and X is quite prominent in Protopterus. He does not describe this nerve as connecting with the branches extending on to the body, but shows its union with the vagus ganglion. This commissure is undoubtedly homologous with the R. recurrens facialis of Batrachus. A few of the sense organs of Protopterus are enclosed in canals, but they are, for the most part, on the surface of the body, as in Batrachus.

8. *Cyclostomes.* — The commissure between the VII and X has been found in Petromyzon and figured by Ahlborn (33). Stannius (34) speaks of the N. lateralis as "*formed partly by a recurrent branch from the facialis passing around outside the auditory capsule, a thing which does not occur in the N. lateralis*

*in higher forms.*" This is a complete description of the course of the R. recurrens facialis in Batrachus.

9. *Amphibia.*—While Batrachus is a true teleost, there are certain superficial resemblances to the Urodeles, the sense organs of both having much the same appearance and arrangement on the body.

As regards the course of the cranial nerves, Strong (11) has pointed out the remarkable homologies that are presented in the tadpole and the teleost; the dorsal branches corresponding to those of teleosts being present in the tadpole but becoming aborted in the adult frog.

## *General Summary.*

*Development of organs and canals.*—The sense organs of the lateral system in Batrachus arise from special cords of cells formed in the lower layer of the epidermis. These cords originate from certain thickenings which make their appearance in the auditory region of very young embryos, and proliferate along definite lines on the head and trunk. The enlarged growing end of one of these cords pushes its way from the auditory region to the extreme posterior part of the body, the swollen appearance remaining conspicuous for some time in the region of the caudal fin.

These thickenings of the ectoderm give rise to the sense organs; each organ arising as a "local proliferation" of cells along the cord (Fig. 15). These cells push through the overlying epidermal cells and gradually take on the form and character of the adult organ, having the hair cells well developed, and the so-called "cupola" structure surmounting the organ.

In a later stage each organ sinks slightly below the surface, and a pointed fold of the skin projects on either side of it. This is the permanent condition of the majority of the sense organs of Batrachus. On each side of the head, however, four short canals are formed. They enclose organs identical with those remaining on the surface, and the canals may be regarded as a fusion and extension of the paired flaps which serve to protect the free organs. In the adult the canals lie in open

channels of the dermal bones and only primary pores are present.

*Innervation.* — The dorsal branches of the VII and X cranial nerves supply the lateral line system. The supraorbital line of organs are innervated by the R. ophthalmicus superfacialis; the infraorbital by the R. buccalis facialis; the operculo-mandibular by the R. mandibularis externus; while the vagus sends branches to the single canal organ in the temporal region, as well as to the organs on the top of the head.

The anterior organs of the trunk are supplied by the R. recurrens of the VII, which forms an anastomosis with a branch of the vagus just outside of, and posterior to, the auditory capsule, and extends on to the body, occupying a position directly superficial to the N. lineae lateralis. The R. recurrens in Batrachus is probably the same as the R. dorsalis recurrens facialis (trigemini) of the Siluroids, or of the cutaneous quinti in Gadus, although following a different course on the side of the body.

It remains for future investigation to determine the exact innervation of the organs on the body of Batrachus.

The complexity of the peripheral nervous system grows more apparent with every step of advance in methods of investigation. In Kupffer's words, "The researches of the last decade in comparative embryology have shown that the development of the peripheral nervous system is a far more complicated process than it was formerly supposed to be" (36). This is especially true in the case of the vertebrate head, as the recent work on Amphioxus by Hatschek (37), and the important investigations by Kupffer (38) on Ammocetes clearly show.

In the views of Hatschek (37) we encounter a slightly modified form of Balfour's hypothesis in regard to the origin of both cranial and spinal nerves from a type of segmental nerves which had only dorsal, yet *mixed* dorsal roots. According to Hatschek the spinal nerves have lost certain elements, while the cranial nerves have retained more of the primitive characteristics. In Petromyzon, Kupffer (38) has shown that the "dorsal spinal nerve root" and the "mixed head nerve root" exist together side by side as coördinate components of a typical head nerve.

In the researches of Kupffer (36), we gain important additions to our knowledge of the development of the cranial ganglia in connection with the thickenings of the ectoderm which have long been recognized, but little understood. Since Beard's (16) and Froriep's (39) simultaneous discovery of "branchial sense organs" in the embryos of sharks, and the corresponding transient structures in embryos of higher forms, there has been much controversy in regard to the question of the ectoblast elements entering secondarily into the formation of the cranial ganglia and nerves. There has been much hesitation on the part of investigators in accepting this fact, for, as Froriep (39) has said, "It would certainly bring about a fundamental change in our views, were we to be convinced that during a long period of embryonic development, the whole ectoblast possessed the capacity to act as 'Nervenkeim.'"

It is now settled beyond dispute that these "placodes" in Ammocetes do furnish material to the processes growing down from the neural ridge, and subsequently forming the cranial ganglia and nerves. The peripheral portion of the "placodes" may become the "foundations of the primary sense organs." The sense organs of the lateral line, although distributed over the entire length of the trunk, are connected with ganglia formed in the head region, and are therefore innervated by cranial nerves. There seems every reason for considering the system as belonging with the more highly specialized sense organs.

In his admirable paper on "The Cranial Nerves of Amphibia," Strong (11) has shown the extensive modification which takes place in the nervous system of Rana, due to the disappearance of the lateral line organs in the adult, and suggests "the importance of taking into full consideration, as a factor, the *cutaneous sense organs* in the attempt to obtain a philosophical understanding of the changes undergone by the peripheral and central nervous systems. The development and specialization of these structures have probably played an important part in the changes leading to the organization of the vertebrate peripheral and central nervous systems."

UNIVERSITY OF CHICAGO,
May, 1896.

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## REFERENCE LETTERS.

*A.* = anal fin.
*ac.b.* = accessory membrane bone.
*ac.md.l.* = accessory mandibular line.
*a.l.* = anterior pit line of head (Amia).
*an.r.* = anterior root.
*an.na.* = anterior nasal aperture.
*ar.* = articular.
*buc.f.* = ramus buccalis facialis.
*cb.* = cerebellum.
*C.H.* = cerebral hemispheres.
*c.l.* = cheek line.
*com.VII–X* = commissure between *VII* and *X*.
*con.st.* = connecting strand.
*d.* = dentary.
*d.b.VII* = dorsal branch of *VII* nerve.
*d.b.ll.n.* = dorsal branch of nervus lineae lateralis.
*d.b.rec f.* = dorsal branch of recurrens facialis.
*d.l.* = dorsal line.
*d.o.l.* = dorsal opercular line.
*ep.* = epiphysis.
*F.R.* = frontal.
*f.g.* = facial ganglion.
*g.l.* = glossopharyngeal nerve (Amia).
*g.g.* = Gasserian ganglion.
*h.l.* = horizontal pit line of cheek (Amia).
*I.O.C.* = infraorbital canal (Amia).
*ll.r.* = lateral line rudiment.
*m.* = muscles.
*MX.C.* = maxillary canal.
*md.l.* = mandibular line.
*m.e.f.* = ramus mandibularis externus facialis.
*m.l.* = middle dorsal pit line of head.
*n.ll.* = nervus lineae lateralis.
*n.c.* = noto chord.
*oc.f.* = supraoccipital foramen.
*oll.* = olfactory lobes.
*OM.C.* = operculo-mandibular canal.
*OP.* = operculum.
*op.f.* = ramus ophthalmicus superfacialis.
*op.l.* = optic lobes.
*O.S.* = opercular spine.
*ot.n.* = otic nerve (Amia).
*p.* = pore of canal.
*P.* = pectoral fin.
*pigm.* = pigment.
*pl.* = posterior pit line of head (Amia).

*p.n.a.* = posterior nasal aperture.
*P.OP.* = preoperculum.
*p.r.* = posterior root.
*rec.f.* = ramus recurrens facialis.
*s.or.* = sense organ.
*SO.C.* = supraorbital canal.
*sp.c.* = spinal cord.
*st.com.* = supratemporal cross-commissure.
*st.v.* = supratemporal branch of vagus.
*T.A.* = tuberculum acusticum.
*T.C.* = temporal canal.
*V.* = ventral fin.
*v.b.rec.f.* = ventral branch of recurrens facialis.
*v.o.l.* = ventral opercular line.
*v.g.* = vagus ganglion.
$v.^2$ = second branch of vagus.
*wd.* = wolffian duct.

## EXPLANATION OF PLATE XVII.

FIG. 1. Side view of head of Batrachus tau, one year old. × 6. Showing the appearance of the lines of sense organs in the adult, also the position of the paired flaps and other projections of the skin on the head.

FIG. 2. Dorsal view of same. × 6.

FIG. 3. Ventral view of same. × 6.

FIG. 4. Dorsal view of skull. Natural size.

FIG. 5. Ventral view of mandible. Natural size. The grooves show the position of the lateral line organs.

In Fig. 4, for *JR* read *FR*.

## EXPLANATION OF PLATE XVIII.

FIG. 6. Embryo of Batrachus at time of hatching, showing the different lines of organs well defined. × 15.

FIG. 7. Side view of head of larva, showing sense organs on the surface. × 6.

FIG. 8. Front view of same.

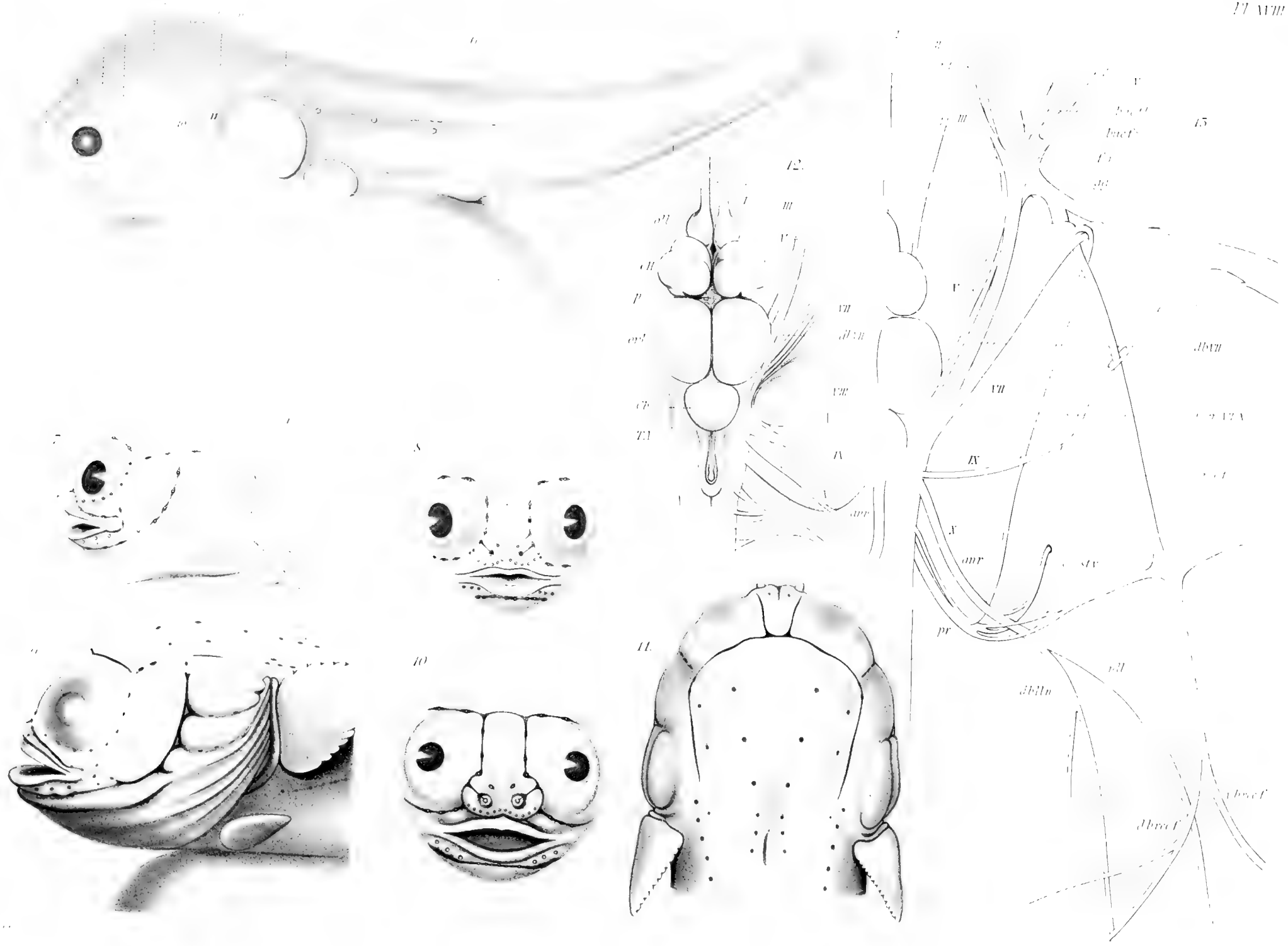
FIG. 9. Side view of head, a few days later, showing canals in process of formation. × 6.

FIG. 10. Front view of same.

FIG. 11. Dorsal view of same.

FIG. 12. Dorsal view of brain, showing roots of cranial nerves. × 6.

FIG. 13. Diagram showing connections between the *VII* and *X*. The intracranial commissure, and the anastomosis of the recurrens facialis with a branch from the *X*. The *VIII* has been omitted, as also the portion of the *X* innervating the branchial region.


12
III
VI
VII
IV
V
IX
X
amr
pr
15
VIII
11
10
8

## EXPLANATION OF PLATE XIX.

FIG. 14. A horizontal section of an Acanthias embryo 17 mm. long, showing the growing point of the lateral line with its "pocket" and the nerve. Cam. Z. 16, oc. 3.

FIG. 15. Drawn from a preparation of the skin, showing the appearance of the cells of the organs at an early stage of their development. Cam. Z. 4, oc. 3.

FIG. 16. A vertical section through a sense organ, showing the relation of the connecting strand to the organ. Cam. Leitz 7, oc. 3.

FIG. 17. Preparation of skin showing the appearance of the rudiment of the lateral line organs with its enlarged growing end. Cam. Z. 4, oc. 3.

FIG. 18. A horizontal section of an embryo of Batrachus 7 mm. long, showing the position of the growing point of the line in its relation to the outer layer of ectoderm and to the muscles. Cam. Z. 16, oc. 3.

FIG. 19. View of same magnified. Cam. Z. 4, oc. 3.

FIG. 20. Cross-section through the enlarged portion of the line. Cam. Leitz 7, oc. 3.

In Fig. 16, for *m* read *n*.

## EXPLANATION OF PLATE XX.

FIG. 21. Diagram showing the innervation of the lateral line organs of Amia. *Journ. of Morph.*, Vol. ii, No. 3, Pl. XLII (reduced).

FIG. 22. Diagram showing innervation of the lateral line organs of Batrachus. The lines in blue indicate the sense organ with the connecting strand.

FIG. 23. Diagram showing dorsal view of the same. The course of the nerves is shown on the right, and the position of the connecting strand, in blue, on the left. The short lines on each side of the organ represent the position of the paired flaps.

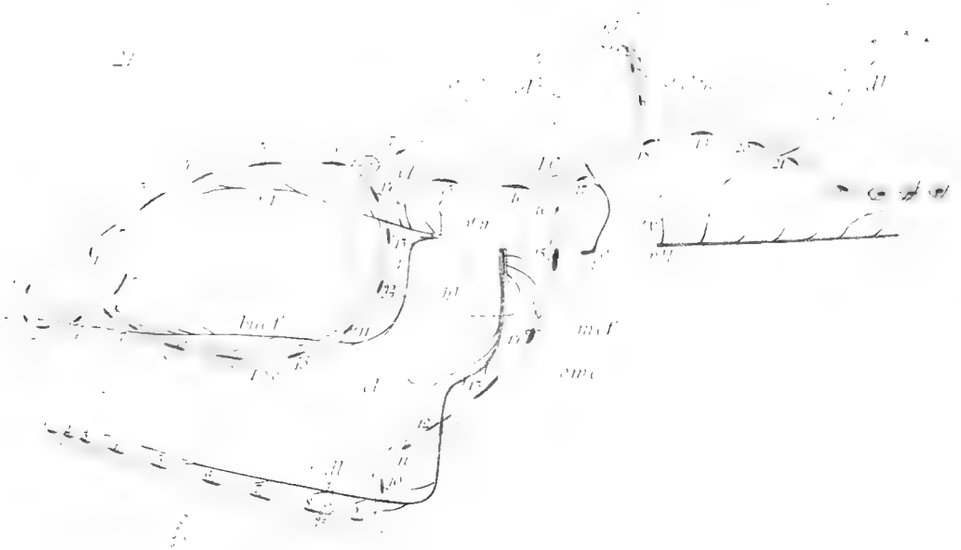
FIG. 24. View of left side of body of Batrachus one year old. Showing position of sense organs in adult. Natural size.

In Fig. 21, for *st.cbm.* read *st.com.*

In Fig. 22, in supraorbital line, supply 1, 2, 3, 4, 5, 6, as indicating the organs of that line.

For *d.b.nec.f.* read *d.b.rec.f.*

For *b.n.ll.* read *d b.n.ll.*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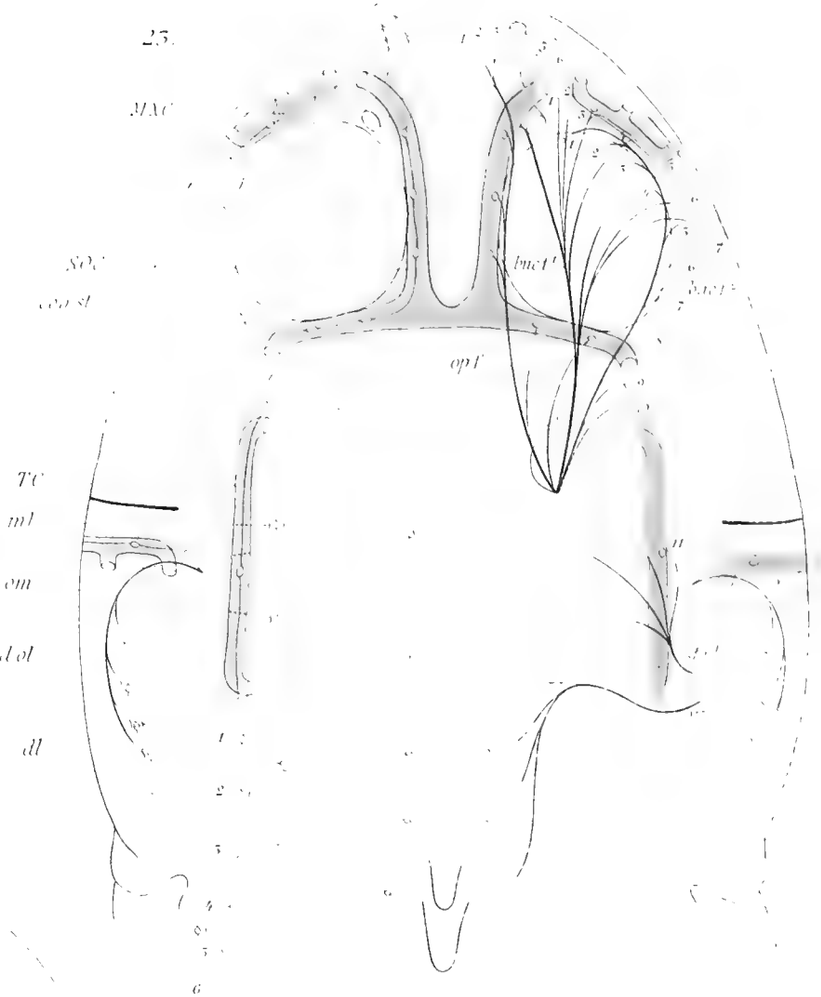
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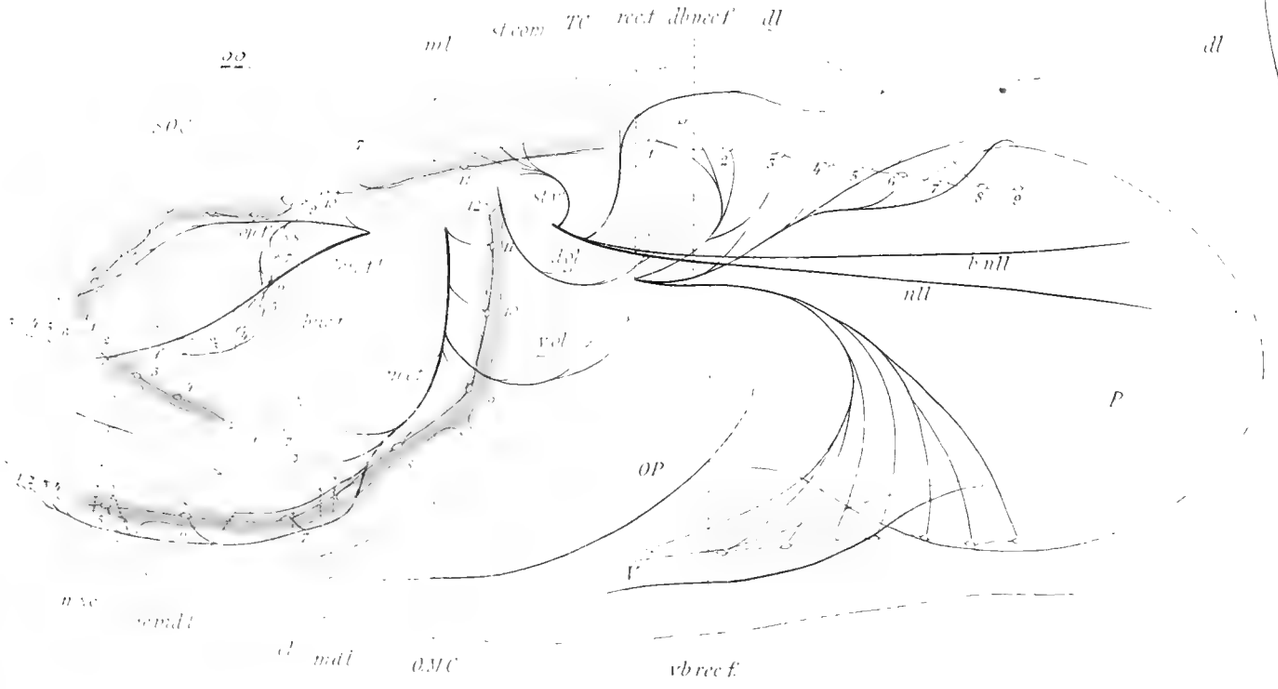
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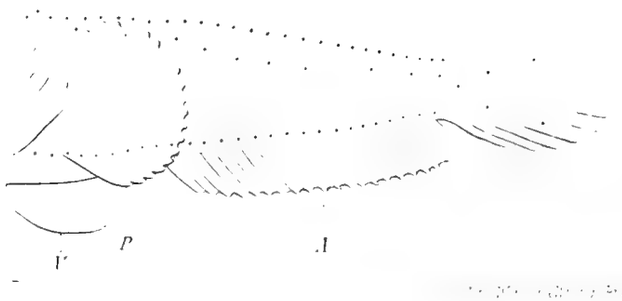
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# COMPARATIVE CYTOLOGICAL STUDIES, WITH ESPECIAL REGARD TO THE MORPHOLOGY OF THE NUCLEOLUS.

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CONTENTS.

## I. INTRODUCTION.

THE following studies are based upon animal cells, both egg cells and somatic cells having been investigated. They were made, primarily, with a regard to the morphology of the true nucleoli (plasmosomes), though numerous other points in ontogenetic cellular development have been considered. In connection with these observations the zoölogical literature upon the subject of nucleoli has been reviewed as thoroughly as possible, and, less completely, the literature from the botanical standpoint as well; reviews are given of these observations of previous writers. No attempt has been made to review the literature from the pathological standpoint. Under the caption "General Comparisons and Conclusions" are compared together the more important deductions from my own observations, and these are compared with those of previous investigators.

The nucleoli are cellular structures which have been studied to much less extent than other constituents of the cell, and though there are numerous observations upon them, these are so scattered through works of more general import that it is well-nigh impossible to collect together all the previous investigations upon the subject. I hope that this explanation may be taken as an apology by any authors whose papers I have chanced to overlook.

At the laboratory of the Fish Commission at Woods Holl, the following species were collected by me: *Montagua*, *Amphiporus glutinosus*, *Tetrastemma catenulatum*, *Zygonemertes*, *Lineus*, *Polydora*, and *Piscicola*. At Sea Isle City, at the laboratory of the University of Pennsylvania: *Tetrastemma elegans*, *Doto*, and certain of the species found at the former locality. *Stichostemma* was collected in the aquaria of the University

of Berlin; and the preparations of the siphonophore *Rodalia* were kindly placed at my disposal by Dr. E. G. Conklin.

*Doto* and *Montagua* belong to the family of the *Aeolidiidae; Amphiporus, Tetrastemma, Zygonemertes,* and *Stichostemma* are *Metanemertini;* and *Lineus* and *Cerebratulus* are *Heteronemertini; Polydora* is a *Polychaete;* and *Piscicola* a rhynchobdellid leech.

The present paper was sent to Dr. Whitman, editor of the *Journal of Morphology*, on Feb. 3, 1897; on receiving the MSS. again in March, 1898, I was able to incorporate in the text reviews of the literature of the whole year 1897. No other changes of importance, however, were then made in the original text, except brief mention of observations which I had made in the past year. It is my intention to follow this paper by others on nucleolar structures, particularly on structures which have received but little consideration in the present paper, namely, "double" nucleoli and chromatin nucleoli.

## II. REVIEW OF THE LITERATURE UPON NUCLEOLI.

In this review shall be considered separately, first, those papers from the zoölogical, and, second, those from the botanical standpoint. The references from zoölogical papers I have endeavored to make as complete as possible, while my citations from the observations of botanical observers are much less numerous, though even in this I have consulted the more important papers from 1880 to the present time. In referring to the zoölogical papers, I have taken them up in chronological order; and in doing so, shall treat separately the periods 1781–1860, 1861–69, 1870–79, and from the year 1880 to the present time I shall treat the literature for each year separately, in order that the reader may more conveniently be able to turn to the citations from a given paper. Under each year papers are reviewed according to the alphabetical sequence of the authors' names. The botanical literature, on the other hand, shall simply be treated in chronological order, without regard to any division into periods. The full titles of the papers referred to are to be found on

page 542, where their arrangement is according to the alphabetical order of the authors' names, both the zoölogical and botanical papers being in this one list. A certain number of contributions dealing with nucleoli are entered into the literature list, which I was unable to find in the libraries at my disposal; all such papers have been distinguished by an asterisk (*); the contents of some of the latter I have reviewed from the citations of other writers.

Literature reviews are here given of all papers, with the object of furnishing a reference library on the subject; in Chapter IV, consequently, brief allusions only are made to the views of particular authors, and readers can compare their views by referring to the present section. This arrangement of the literature appears the most practical.

### *A.* ZOÖLOGICAL LITERATURE.

#### *1781–1860.*

Fontana (**1781**, cited by Carnoy, '**84**) was the first to figure the nucleolus in the nucleus, which he describes as "un corps oviforme, pourvu d'une tache en son milieu."

The discoverer of the nucleolus in germinal vesicles is R. Wagner ('**35**), and he termed it "Keimfleck" or "macula germinativa." He notes that the germinal vesicle of *Unio* and *Anodonta* "zeigt constant zwei Flecke in Form von Kreisen, welche sich schneiden, selten finden sich Abweichungen; der grössere derselben möchte eine gewisse Aehnlichkeit mit dem Keimfleck haben." In his "Nachtrag" to the same paper, he states: "Der Keim ist bei seinem ersten Auftreten eben das, was ich Keimfleck genannt habe. Es ist eine Schicht körniger Masse, welche bald einfach (Säugethiere, Schnecken, Insekten etc.) als Fleck erscheint, bald mehrere zerstreute Kügelchen bildet (Flusskrebs, Fische, Batrachier), . . . die an der inneren Wand des Keimbläschens angeheftet sind." In two subsequent communications ('**36**, '**37**) he notes the occurrence of nucleoli in the germinal vesicles of *Coryna*, *Lucernaria*, *Cyanea*, *Chrysaora*, *Asterias*, and *Insecta*, and finds in *Melolontha vulgaris* one large and one small nucleolus. Finally he remarks: "Viel-

leicht bildet das Material des Keimbläschens und der Keimflecke die Grundlage zum serösen Blatt und zum Fruchthof der Keimhaut." (Jones, '35, '37, does not mention the nucleolus; accordingly, he is not the discoverer, as is claimed by Bischoff.)

Valentin ('36, cited by Carnoy, '84) describes the nucleolus as a "rundes Körperchen, welches eine Art von zweitem Nucleus bildet." (On this historical ground Carnoy considers the term "nucleolus" should be limited to his "nucléole-noyau.")

Valentin ('39, mentioned by Carnoy, '84) introduces the terms "nucleolus" and "Kernkörperchen"; the latter term was proposed also by Schwann ('39) in the same year.

Bischoff ('42) found in the egg of the rabbit one nucleolus, "ein schwach granulirtes Körnchen," which he considers to be a "Zellenkern."

Vogt ('42) found several nucleoli (six to twelve) in the ova of *Coregonus;* these subsequently migrate into the yolk to form the first cells of the blastoderm.

Leydig ('49) describes in the germinal vesicle of *Nephelis* one nucleolus, in *Clepsine* one or numerous ones, in *Piscicola* two to four, while in *Haemopis* "der Keimfleck war einfach, 8-förmig oder doppelt."

Kölliker ('49) studied numerous Gregarines, and concludes that the nucleoli ("Körnchen") "bei manchen Gregarinen gewisse bestimmte Entwickelungen durchlaufen, nämlich bei jungen Individuen einfach vorhanden sind, bei älteren allmälig in zwei, drei oder mehr Körner zerfallen." In *G. terebellae, clavata, saenuridis,* and *enchytraei* there is a single nucleolus; *G. sipunculi* has from one to six; *G. heeri,* six to eighteen; *G. sieboldii,* one to seven, which are either homogeneous or vacuolar, or else only one or two are present, and each of these is composed of a mass of smaller ones; *G. brevirostra* has from six to nine nucleoli.

Lovén ('49) studied the eggs of *Modiolaria, Cardium, Patella,* and *Solen,* and found that during the process of fecundation the nuclear membrane ruptures, and the nucleolus passes out through the vitelline membrane. (It is very probable that he confused the nucleolus with a pole body.)

Quatrefages ('49) found that preceding the first maturation division of *Teredo* the nucleolus dissolves in the nucleus.

v. Wittich ('49) found that in the germinal vesicles of *Lycosa, Theridium, Epeira* the "Keimfleck" first appears "als ein matt gelblicher, nicht immer scharf begrenzter, aber durchaus homogener Fleck, wird immer entschiedener rund, verliert seine Homogenetät, indem er hie und da den Schein von unregelmässig rundlichen Aushöhlungen bietet, und neben ihm treten zuletzt zerstreut ungleich geformte Körperchen auf, die dem ersteren sehr ähnlich, an Zahl immer mehr zunehmen, je mehr sich das Bläschen [Kern] seinem gänzlichen Schwinden nähert." In *Gasterosteus aculeatus* the number of the "Keimflecke" increases with the size of the egg. In the youngest germinal vesicles of *Fringilla* there is at first no nucleolus, later a single large, excentric one.

Leydig ('50) finds that in the ovarial egg of *Paludina vivipara* there are two widely separated nucleoli, while in the ripe egg they are in contact with each other: "so muss wohl angenommen werden, dass der achterförmige Keimfleck des reifen Eies durch Aneinanderrücken und theilweises Verschmelzen der früher getrennten Körperchen entstanden sei."

Leydig ('52), ovum of *Synapta digitata:* there is a single nucleolus with a vacuole; "was aber als eigenthümlich hervortritt, ist, dass er constant an einem Pol des Keimbläschens liegt und zwar in einer tellerförmigen Grube desselben."

Leuckart ('53) states: "Der Keimfleck bildet eine zusammenhängende Masse von feinkörniger Beschaffenheit und opakem Aussehen, die unter dem Deckgläschen mancherlei Formen annimmt und ohne Umhüllungshaut ist. Nicht selten lassen sich im Innern auch einzelne grössere Moleküle ganz deutlich unterscheiden. In manchen Fällen nehmen diese Moleküle an Zahl und Selbständigkeit in einem solchen Grade zu, dass der ganze Keimfleck eine haufenförmige Aggregation von Körnern darstellt."

Hessling ('54) finds in the youngest eggs of *Unio* a single large nucleolus; in larger ova there is a larger and a smaller nucleolus, the latter having divided off from the former, and showing a different reaction to acetic acid.

Lacaze-Duthiers ('54) finds in the eggs of *Lamellibranchs* either one nucleolus, or when two are present they are of unequal size.

Leydig ('55a) says that in the egg of *Cyclas* "der Keimfleck hat constant die Bisquitform." In a second paper of the same year ('55b) he makes the following notes on the ova of *Rotatoria:* in *Notommata myrmeleo* there are about 100 finely granulated nucleoli; in *N. sieboldii* "Die Keimflecke erscheinen als Haufen von kleinen, hellen Kügelchen," and disappear in the ripe egg; in *N. centrura* and *Brachionus bakeri* there is a single large nucleolus.

Agassiz ('57) in studying the egg of the turtle introduces the following terms: "ectoblast" for cell membrane, "mesoblast" for nucleus, "entoblast," or "Wagnerian vesicle," for the nucleolus, and "entosthoblast," or "Valentinian vesicle," for the body sometimes enclosed in the latter. In the youngest ova the nucleoli are absent, later they become numerous and large, though they disappear in the ripe egg. The excentric vacuole ("Valentinian vesicle") of the nucleolus "increases in size at a greater proportionate rate than its parent, the "Wagnerian vesicle," till at its final stage it oftentimes occupies three-fifths of the diameter of the generating medium."

Lacaze-Duthiers ('57), ovarian egg of *Dentalium:* at first there is but a single nucleolus, later a second one appears and apposes itself to the former; the volumes of the two are different. (*Cf.* Fol, '89.)

Remak ('58), blood cells of *Gallus:* "Es kann kaum einem Zweifel unterliegen, dass die Theilung der Blutzellen mit der Theilung des Kernkörperchens beginnt. . . . Die Regel ist, dass das Kernkörperchen sich in zwei Theile abschnürt, und ebenso der Kern in zwei Kerne. Wie es aber zuweilen vier Kernkörperchen giebt, so finden sich auch zuweilen vier Kerne in einer Zelle."

*1861–69.*

Pflüger ('63) found one nucleolus in the egg of the calf. While in the "Urei" of the cat he makes the interesting observation that after a division of the nucleus, whereby one

of the daughter-nuclei retained the original nucleolus, in the other a new nucleolus soon appeared, first in the form of a granular mass.

In the paper by Balbiani ('64) movements of nucleoli are described for the first time, and these observations were made upon the living eggs. The first kinds of movements which he distinguishes are exhibited by the eggs of spiders: "ces mouvements de la tache germinative sont caractérisés par la production de prolongements transparents ayant presque toujours la forme de lobes arrondis qui s'allongent et se rétractent alternativement." The second kind of movements is shown in the egg of *Phalangium*, where there is a single large, spherical nucleolus, which appears spongy, owing to the presence of a number of vacuoles, some of which "s'élèvent plus ou moins au dessus de la surface en soulevant sous forme d'une ampoule la couche la plus externe de la substance du corpuscule. . . . Lorsqu'un porte son attention sur une de ces vésicules superficielles, on ne tarde généralement pas à la voir grossir insensiblement, en même temps que la couche de substance qui forme sa paroi extérieure se soulève en s'amincissant de plus et plus ; puis, assez brusquement, cette paroi se rompt comme sous la pression d'un liquide intérieur, et ses bords se rétractent vers la base adhérente de l'ampoule qui se trouve ainsi transformée en une petite cupule ou excavation superficielle, . . . et bientôt il ne reste plus aucune trace de l'ampoule ni de l'excavation qui lui a succédé." All the peripheral vacuoles discharge themselves thus in succession, while at the same time the smaller central vacuoles increase in size and wander towards the periphery to take the place of the preceding. Balbiani compares these movements to those of the contractile vacuoles of the *Rhizopoda*, but notes this difference: in the latter forms the vacuole always forms itself at the same place again. In the eggs of *Geophilus* and of *Helix pomatia* he finds that the vacuole discharges through a small orifice.

Balbiani ('65b) describes some remarkable structures in germinal vesicles, all studied in life. In *Geophilus longicornis* there is an external infundibular canal extending from the sur-

face of the nucleus to the surface of the vitellus, its larger opening being apposed to the nucleus. A smaller inner infundibular canal extends from the nucleolus into the outer canal. The numerous vacuoles of the nucleolus are contractile, and empty into the inner canal. He believes that these canals disappear at the time that the nucleolus does. "Dans les ovules de la Chienne, après la séparation des follicules primordiaux, la vésicule et la tache germinative offrent chacune un prolongement canaliculé dont l'un est intérieur à l'autre, comme chez le Géophile. . . . Chez la Raie, où les ovules renferment généralement d'un à quatre petits corpuscules germinatifs creusés d'une vacuole centrale, chacun de ceux-ci émet un nombre variable de petits canaux, ordinairement de deux à quatre, lesquels traversent dans différentes directions la cavité de la vésicule, percent sa paroi et vont se perdre dans le vitellus ambiant. . . . Chez les poissons osseux et les Batraciens, dont les œufs renferment . . . un grand nombre de taches germinatives adhérentes à la paroi interne de la vésicule, celle-ci est entourée d'un système de canaux rayonnants vers la surface de l'œuf, légèrement flexueux et de longuer inégale suivant le trajet qu'ils ont à parcourir pour atteindre cette surface. Chaque canal est en rapport avec un des corpuscules précédents, et présente un calibre correspondant au diamètre de ce dernier. . . . Quelquefois, ainsi que je l'ai observé chez quelques Crustacés (Ecrevisse, Cancer moenas), ces taches multiples s'ont paru en outre réunies, dans l'intérieur de la vésicule, par des canaux qui s'etendaient de l'une à l'autre. . . . Chez plusieurs Annélides, Turbellariés, Mollusques et Acalèphes, dont j'ai examiné les œufs, ceux-ci ne renfermaient pour la plupart qu'une tache germinative simple, souvent assez volumineuse, en rapport avec un canal unique renfermé dans l'intérieur d'un deuxième canal émanant de la vésicule germinative." In the germinal spots of *Helix*, *Vortex*, and *Prostomum* he noticed one or several contractile vacuoles.

Schrön ('65) finds in the eggs of the cat and rabbit one or two "Körner" in the nucleoli of the larger eggs, though not in those of the smaller eggs. He considers the "Korn" dif-

ferent in structure and substance from the rest of the nucleolus, and that it is characteristic for a certain stage of the cell.

Stepanoff ('65) describes for the youngest germinal vesicles of *Cyclas* two nucleoli which are unequal in size, while in more mature ova there are usually two (seldom one) large ones. He figures, further, in one nucleus a smaller nucleolus in contact with a larger one.

La Valette St. George ('66) studied in iodized serum the germinal vesicles of various animals. In the egg of the kitten there is one large nucleolus, either homogeneous or finely granular, containing sometimes a large vacuole. In that of the embryo of a sheep he noticed one or several nucleoli, with slight differences in size, finely granular in structure, and containing each a clear vacuole. In the egg of a larva of *Libella* there was a small and a large nucleolus, the latter being darker and more refractive, and spherical or irregular in form; "seine Substanz war entweder homogen oder zeigte je nach der Einstellung des Mikroskopes hellere oder dunklere Flecken von sehr verschiedener Zahl und Grösse, von unmessbarer Kleinheit bis zu zwei Drittel des Keimfleckes. . . . Anfangs war der grosse Keimfleck unregelmässig geformt fast viereckig und zeigte in der Mitte eine hellere Stelle, etwa ein Drittel so gross wie der ganze Keimfleck und daneben ein zweites kleineres Fleckchen. . . . Nach einer Viertelstunde hatte er seine Form geändert, der kleinere Fleck war verschwunden, der grössere nach der Spitze zu gerückt. Nach Verlauf einer halben Stunde war er kuglig geworden und jene helle Stelle verschwunden." (In this last stage the nucleolus touches the nuclear membrane, according to his Fig. 2c.) In the egg of *Porcellio scaber* the nucleolus is an irregular granular mass, and later becomes a massive body; "zuweilen stellt er einen nach einer Seite geöffneten Ring dar, oft auch eine ausgehöhlte Kugel." By these observations he believes he has proved what Schrön termed a solid granule ("Korn") to be a vacuole.

Ransom ('67), egg of *Gasterosteus:* young eggs with numerous peripheral germinal spots, which are spherical and homogeneous. He supposes these "are soluble in some of the constituents of the yolk, and we may thus explain their disap-

pearance in ripe ova." A 1.5% solution of NaCl gives rise to vacuoles in the nucleoli (this antedates the observation of Morgan, '96).

Van Beneden ('69) studied *Gregarina gigantea:* "Le nombre de nucléoles varie à chaque instant ; quelques-uns disparaissent, tandis que d'autres se forment ; ils apparaissent sous forme d'un petit point presque imperceptible ; ce point grandit jusqu'à certaines limites ; il devient un véritable corpuscule formé d'une substance homogène très-réfringente, puis le corpuscule diminue de volume ; il réfracte de moins en moins la lumière, enfin il disparaît."

Claparède ('69) found in the egg of *Lumbricus terrestris* that the nucleolus "ist doppelt, indem er aus zwei einander berührenden ungleich grossen Kügelchen besteht."

*1870–79.*

Eimer ('71), epithelial cells of the snout of *Talpa:* each nucleolus is surrounded by a clear space ("Hof"), and the outer boundary of this space "war bezeichnet durch zahlreiche kleine Pünktchen. . . . Im optischen Querschnitt stellten diese Körnchen einen Kreis um den hellen Hof des Kernes dar."

Eimer ('72) finds in the earlier stages of the egg of *Lacerta* that all the nucleoli are grouped near the center of the nucleus, while in more advanced ova there are numerous larger peripheral nucleoli, and smaller ones in the other portions of the nucleus ; around each of the large peripheral nucleoli are situated concentric rows of smaller ones. Here, as well as in *Cistudo*, *Testudo*, and *Tropidonotus*, the smallest nucleoli are homogeneous, while the larger contain vacuoles. He concludes that "die complicirt gebauten Keimflecke aus einfachen Körnchen" are built up.

Kleinenberg ('72) : in the egg of *Hydra* the single spherical nucleolus contains "ein auffallend stark lichtbrechendes Körperchen. . . . Nach kurzer Zeit schwindet es wieder." The nucleolus then becomes irregular in form, breaks into small granules, and he supposes that these latter become dissolved.

Eimer ('73), nervous system of *Beroë:* each nucleus contains one large nucleolus. "Aufmerksamer Beobachtung kann es nicht entgehen, dass jede Epithelzelle von einer Primitivfibrille versorgt wird. . . . Ich kann nur so viel sagen, dass ich dieselbe [Primitivfibrille] stets auf das Centrum des Kerns zugerichtet sah, so dass ich zu der Ansicht hinneige, es werde sich späterhin ihre Endigung im Kernkörperchen feststellen lassen."

Fol ('73) noticed in the egg of *Geryonia fungiformis* one large nucleolus, containing one large, or several smaller vacuoles.

Auerbach ('74). This important paper I have been unable to consult in the original, and quote from citations by R. Hertwig ('76) and Flemming ('82). According to Auerbach the nucleus is originally a vacuole in the protoplasm, around which a layer of the latter becomes differentiated to form a nuclear membrane. In this vacuole a nucleolus appears later, being derived from the protoplasm, either by a separation of particles from the nuclear membrane or is produced out of those protoplasmic particles which had penetrated from the protoplasm into the original vacuole. He distinguishes "enucleolar," "uninucleolar," and "multinucleolar" nuclei, the first being the more primitive state. The nucleolus has the value of an elementary organism: as long as it is homogeneous, it is comparable to a cytode; when a vacuole appears in it, the latter stands in the same relation to the nucleolus as this does to the nucleus, so that that vacuole may be considered the nucleus ("Kern") of the nucleolus. The original single nucleolus can divide into numerous nucleoli, and the latter, by the disappearance of the nuclear membrane, become free, and each develops into a separate cell. Auerbach considers this theory as "eine vorläufige, noch mit Vorbehalt aufzustellende und weiter zu prüfende."

A. Brandt ('74) observed in life (in the blood fluid) slow amoeboid motions of the single nucleolus of the egg of *Blatta.*

Flemming ('74) investigated the egg of *Anodonta.* In young eggs the nucleolus consists of two apposed spheres of equal diameter; in larger eggs one of these spheres is much larger

than the other. "Der kleinere Theil ist stärker lichtbrechend, auch etwas stärker tingirbar, und beim Zerdrücken resistenter als der grosse: beide zeigen sich hierbei als eine homogene, zähe Masse." The smaller has usually one large vacuole; the larger has several smaller vacuoles. "Bei Anodonta scheinen mir ausserhalb der Fortpflanzungszeit die beiden Theile normal zusammenzuhängen. . . . Kurz vor Eintritt der Befruchtungszeit gewahrt man viele (aber nur reife, grosse) Eier, an deren Kernkörpern eine wirkliche Trennung vorgegangen ist; aber in der Art, dass der kleinere Bückel stückweise abgesprengt wird."

Haeckel ('74) notes in the nucleolus of some egg cells "ein innerstes Pünktchen, einen Nucleolinus, welchen man Keimpunkt (Punctum germinativum) nennen kann. Indessen haben diese letzteren beiden Theile (Keimfleck und Keimpunkt), wie es scheint, nur eine untergeordnete Bedeutung," only the yolk and the nucleus being of fundamental importance.

Ludwig ('74) gives notes on the number of nucleoli in various germinal vesicles. In the *Coelenterata* "Das Keimbläschen umschliesst durchgängig einen einzigen Keimfleck, welcher häufig nochmals ein Körnchen beherbergt." There is one germinal spot in *Echinus*, *Amphidetus*, *Solaster*, *Branchiobdella*, and in *Trematodes* and *Rhabdocoeles*.

Van Beneden ('75) remarks in regard to the egg of the rabbit, that there is one nucleolus, and "deux ou trois petits corps arrondis qui j'ai appelés pseudonucléoles." When the nucleus, during the maturation of the egg, has reached the "zone pellucide" of the yolk, "le nucléole s'accole à la membrane de la vésicule du côte de la surface de l'œuf, là où la vésicule est appliquée contre la membrane. Il s'aplatit contre la membrane et se soude avec elle; sa substance plastique s'étale en une plaque qui présente d'abord un épaississement médian. Cette lame je l'ai appelée plaque nucléolaire." Shortly afterwards the latter body "grâce probablement à la contractilité inhérente à sa substance, . . . se ramasse en un corps de forme variable, souvent ellipsoidal, quelquefois lenticulaire ou en forme de calotte, que j'ai appelé le corps nucléolaire." The latter is the first pole body ("corps directeur"), the nucleoplasm plus the pseudonucleolus constituting the second.

Eimer ('75) studied the egg cells of *Silurus* in eye fluid, and found the nucleolus to present amoeboid movements.

Kidd ('75) found slow amoeboid movements of the nucleoli of the epithelial cells from the mouth of the frog. These cells were placed in humor aqueus, and studied on a stage heated to 39° C.

A. Schneider ('75) says: "Les nucléoles ne sont pas un elément constant de la structure des Grégarines; beaucoup d'espèces en sont normalement privées. Dans les genres *Clepsidrina*, *Euspora*, *Gamocystis*, il n'y a jamais qu'un nucléole, permanent, très-volumineux et sphérique. . . . Dans tout ces genres, jamais deux individus ne sont semblables à eux-mêmes au point du nombre, de la grandeur, de la configuration, de l'opacité ou de la transparence de leurs nucléoles."

F. E. Schulze ('75) noticed in life that an equal division of the nucleolus precedes that of the nucleus, in *Amoeba polypodia*.

Auerbach ('76) repeats some of his previous observations ('74) and adds that the nucleoli show a further similarity to the cytoplasm, in that they have a tendency to produce vacuoles.

Balbiani ('76) describes certain structures in the egg of *Stenobothrus*, which may be chromatic filaments, though I may give a brief citation in regard to them in this place. The contents of the nucleus in the fresh state appear "rempli de petites hachures pâles, tantôt paralleles les unes aux autres, tantôt distribuées plus ou moins irrégulièrement dans la cavité nucléaire. . . . À l'aide de l'acide acétique, on s'assure que ces hachures sont déterminées par les corpuscules en forme de bâtonnets étroits . . . chaque bâtonnet paraît formé de petits globules réunis en série." At the time of nuclear division, these "bâtonnets" become less numerous but larger.

Van Beneden in the same year ('76) gives the results of observations on the egg of *Astéracanthion*. There is one large nucleolus, and eight to fifteen small "pseudonucléoles." He did not notice amoeboid motions in these, but found change of form and successive re- and disappearance of the nucleoli in *Rana*, *Polystomum*, *Gregarina*, and *Monocystis*. "Mais je ne doute

pas que les différences constatées dans la forme de la tache germinative ne doivent être attribués à la contractilité de la substance des nucléoles." The vacuoles in the nucleoli are probably "le résultat de l'union momentanée de certaines parties de la substance nucléolaire avec le suc nucléaire." Before its disappearance the nucleolus breaks into fragments, which then dissolve in the "substance nucléaire." In this fragmentation one fragment is always larger than the others, and contains the vacuole of the primitive nucleolus; it persists until all the smaller fragments have disappeared.

Bütschli ('76) found that the nucleolus disappears before the formation of the first pole spindle in *Tylenchus*, *Anguillula*, *Notommata*, *Brachionus*, *Triarthra*, *Aphis*. He mentions that von Siebold, in 1848, first introduced the name "nucleolus" for the micronucleus of the *Infusoria*, and compared it with the nucleoli of metazoan cells. He also cites some of the earlier writers who compared the pole bodies with nucleoli.

O. Hertwig ('76) calls the nucleolus "das wichtigste Formelement des Kerns," and terms its substance "Kernsubstanz" in opposition to the "Kernsaft" (compare his brother's paper of the same year). In the process of maturation he holds that "der Eikern der aus dem Keimbläschen frei gewordene oder ausgewanderte Keimfleck ist." He noticed vacuoles in, but not amoeboid movements of, the germinal spot of *Toxopneustes lividus;* he observed such motions, however, in the germinal spots of *Rana* and *Pterotrachea*.

R. Hertwig ('76) terms the dense substance of the nucleolus "Kernsubstanz." "Entweder leiten sich die vielen Kernkörper direkt aus dem homogenen Zustand des Kernes ab, indem die Aussonderung der Kernsubstanz an verschiedenen Punkten gleichzeitig begonnen hat; oder die zahlreichen Nucleoli sind, . . . durch Theilung aus einem ursprünglich einfachen Nucleolus entstanden." He believes that the "Nucleoli die Träger der Kernfunction sind. . . . Somit müssen wir in allen den Fällen, in denen sich ein oder mehrere Nucleoli im Kerne differenziren, in diesen die Thätigkeitscentren des Kernes erblicken."

Schwalbe ('76) studied the nuclei of retinal ganglion cells of

the ox, rabbit, and sheep: in the smallest nuclei there is no nucleolus within the nucleus, but there are small peripheral prominences on the inner surface of the nuclear membrane; when a nucleolus is present within the nucleus it is jagged in outline, with fine, thread-like processes. The substance of the nuclear membrane "stimmt in allen Eigenschaften mit der des Kernkörperchens vollständig überein, und ist mit ihr continuirlich." Further, the substance of the peripheral prominences is quite identical with that of the nucleolus, and "Man könnte in dem Falle, wo ein innerer Nucleolus fehlt, geradezu davon reden, dass als Ersatz dafür wandständige Kernkörperchen vorhanden seien." In similar cells of the calf, there are no nucleoli in the smallest nuclei; in larger ones there are from two to four, one or two lying within the nucleus, the others being mere thickenings of its membrane; "beim Wachsen des Kernes (12.5$\mu$) nimmt die Höhe und Zahl dieser Wandverdickungen immer mehr ab, während im Innern ein gut ausgebildeter zackiger oder eckiger Nucleolus von 2.7 bis 3.6$\mu$ das gewöhnliche ist." He considers the substance of the nucleoli and of the nuclear membrane to be at first identical, and to be diffused in the "Kernsaft." In the sympathetic ganglion cells of the frog, he noticed, on the heated stage, that the nucleoli exhibited slow changes of form; and in these nuclei he distinguishes "Nucleolarsubstanz, den Kernsaft und die reticuläre Substanz."

O. Hertwig ('77a) found in the egg of *Haemopis* one large nucleolus, with usually one large vacuole; and also a number of small nucleoli, some of which contain each a small central vacuole. In the production of the pole bodies: "Aus den Theilstücken des Nucleolus und einem Rest des Kernsaftes entsteht ein fasriger, spindelförmiger Kern . . . es muss dahingestellt bleiben, ob der ganze Nucleolus oder nur ein Theil desselben und ob die Nebenkügelchen [Nebennucleolen?] in die Zusammensetzung der Spindel mit eingehen."

v. Kennel ('77) remarks of the ripe egg of *Malacobdella*: "der Kern . . . enthält eine mehr oder minder grosse Anzahl stark lichtbrechender runder Tröpfchen, die sich meist an seiner Peripherie befinden."

Mark ('77) : the salivary gland cells of *Chionaspis* contain each forty to fifty nucleoli; corresponding cells of *Aspidiotus* have a single large one which may contain from two to seven "nucleoluli." In cells of the oval gland of *Chionaspis* the nucleus contains a true nucleolus, usually without nucleoluli, and also a "Fetttröpfchen," which differs from the former in color and refraction. (The Fig. 32 of the salivary gland cells of *Aspidiotus* shows each nucleus to contain a double nucleolus, containing a larger sphere apposed to, in one case separate from, a smaller colorless sphere.)

A. Brandt ('78) gives observations on the germinal vesicles of different forms. In *Aeschna grandis :* "Der vom Keimbläschen umschlossene Keimfleck ist wie dieses, ursprünglich rund, aber in noch viel höherem Grade, und zwar unstreitig bei allen von mir beobachteten Insekten, amöboid beweglich, so dass seine Form meist sehr verschieden erscheint. Nicht selten ist er in einige Theile zerfallen. . . . In einzelnen Keimbläschen lagen ausser dem Keimflecke noch ein oder mehrere Körnchen von verschiedener Grösse ;— nur ein Paar Keimflecke wurden aufgefunden, welche anscheinend aus zwei aneinandergedrängten und theils übereinander geschobenen Kugeln bestanden." In *Periplaneta* vacuoles as well as solid "secundäre Keimflecke" occur in the nucleolus. In the egg of *Nemura*, after the action of acetic acid, the vacuoles in the nucleolus increase in size and in each a small granule is to be seen. In *Gryllus*, *Lepisma*, and *Holostomis* the germinal spot is amoeboid : "Die amöboide Beweglichkeit veranlasst nicht selten das Loslösen einzelner Partikel, welche, wie der Keimfleck selbst, amöboid-contractil sind. Die Zahl und Grösse dieser gelegentlich wieder zusammenfliessenden Partikel ist eine äusserst. verschiedene"; thus the nucleolus may break into a number of equal-sized pieces, or into a mass of very fine granules. In the egg of *Tegenaria* there is usually a single vacuolated nucleolus, though sometimes there may be present also two "Nebenkeimflecke." In *Distomum* the "Keimfleck . . . ist in sehr hohem Grade mit amöbenartiger Beweglichkeit begabt," and there is a central body in the nucleolus which changes its form periodically. Brandt observes

in regard to the frog's egg : "Der Keimfleck des Froscheies, in den allerjüngsten Eianlagen meist ein zusammenhängendes Gebilde, erscheint bekanntlich später, in eine grössere Anzahl von rundlichen Klümpchen zerfallen — und diese fand ich (bei Rana esculenta) amöboid gestaltet"; and adds, against Bütschli ('76), "ist einzuwenden, dass dieser Zerfall des Keimflecks als amöboide Erscheinung keineswegs auf ein Absterben, sondern im Gegentheil auf eine erhöhte Lebensthätigskeit hinweist."

Brock ('78) : the immature ovum of *Anguilla* has one or two large nucleoli ; the number of the latter increases with the size of the egg.

Eimer ('78) notes the great relative and absolute size of the nucleus and nucleolus in ganglion cells, and finds it to be paralleled only in egg cells.

O. Hertwig ('77b, '78a) noticed in the nucleolus of the maturing egg of *Asteracanthion* certain changes, "die darin bestehen, dass die in seinem Innern bisher zahlreich vorhandenen kleinen Vacuolen verschwinden und in seiner Mitte oder mehr der Peripherie genähert eine grössere Vacuole erscheint, die fast ganz von einem kugligen aus Kernsubstanz bestehenden Körper erfüllt wird. . . . Plötzlich verschwinden die in ihm gelegenen Vacuole mit ihrem kugligen Körper unter dem Auge des Beobachters," and in consequence the nucleolus begins to gradually shrink in size, and 1½ hours afterwards has completely disappeared. The body within the large vacuole of the nucleolus corresponds to the smaller, more deeply staining portion of the original nucleolus, and during the nuclear division reaches out of an opening in the vacuole beyond the surface of the nucleolus, takes on the form of a long, thin rod, and occupies the middle point of the first pole spindle; while at the same time the remaining portion of the nucleolus gradually breaks into a granular mass, which then disappears. Also in *Sphaerechinus*, *Ascidia*, some *Coelenterata*, and various *Mollusca*, he noticed a similar differentiation of the nucleolus into two substances, namely, a smaller, deeply staining portion apposed to, or enclosed by, a lighter, larger portion.

O. Hertwig, in still another paper ('78b), investigated the

germinal vesicles of various animals. In *Eucope polystyla* there is one nucleolus in small eggs, several in riper ones: "Es liess sich hier feststellen, dass die zahlreichen Nucleoli durch Ablösung vom ursprünglichen einfachen Keimfleck entstehen."

Klein ('78) studied the stomach cells of the newt, and concludes "that in most cells the so-called nucleoli are local accumulations of the intranuclear network, that they are inconstant in size and number, and that they are only transitory appearances."

Schindler ('78), Malpighian tubules of insects: after a cell has become obliterated by the outflow of its secretion, its nucleus becomes a new cell, and its nucleolus a new nucleus.

Whitman ('78) found in the egg of *Clepsine* one to three nucleoli, each "composed of several highly refractive pieces."

Bergh ('79) found in the egg of *Gonothyraea* (*Campanularia*) a single large nucleolus, which is usually round, but sometimes with irregular outlines caused by slow amoeboid movements (observed in life), these motions being most vigorous later, when the nucleolus begins to divide. It increases in size, and acquires one or two vacuoles. In a later stage, but before the production of the pole bodies, there are a number of irregular nuclear bodies (staining as the original nucleolus), which had been produced by division of the nucleolus; in one case he actually observed the division of the nucleolus, which lasted half an hour, and at the same time the vacuole of the primitive nucleolus seemed to divide into two, so that each daughter-nucleolus received a daughter-vacuole. "Oft macht es den Eindruck, als ob das Volum der secundären Keimflecke zusammengenommen grösser wäre, als das der primären für sich . . . eine active Wanderung der Nucleoli durch den Kernsaft, wie dies Auerbach ['74] bei gewissen Nematoden in den Vorkernen gesehen hat, kommt wahrscheinlich hier nicht vor." The nucleolus also divides in the egg of *Clava*. In the eggs of *Psammechinus* and *Echinocardium*, the single nucleolus begins to fragment before the chromatic network has disappeared. The *Phallusia* egg contains one large germinal spot, which probably disappears without fragmenting: "ich habe nämlich unter Eiern, die im Keimbläschen einen scharf

begrenzten, durch die Osmium-Carminbehandlung rubinroth gefärbten Keimfleck zeigten, auch solche gefunden, welche statt dessen eine sehr feinkörnige, bisweilen rubinroth, bisweilen weniger intensiv rothgefärbte Masse enthielten, die nicht scharf contourirt war, aber von derselben Grösse wie der Keimfleck. Falls diese Deutung, es schwinde der Keimfleck ohne sich vorher zu theilen, richtig ist, beginnt die Auflösung desselben mit dem Schwinden der Vacuolen in seinem Innern."

Klein maintains his previous views in regard to the nature of the nucleoli in two papers published in the following year ('79a, '79b).

*1880.*

Van Beneden ('80) studied the egg of the bat, and found one nucleolus (rarely two): "on trouve en outre quelques granules très petits, tous d'égales dimensions, répandus dans le corps de la vésicule (pseudonucléoles)"; the latter have no resemblance to any part of the chromatic filament.

Bütschli ('80) incorporates in his great "Protozoenwerk" the observations of preceding authors. In *Hyalosphenia* there may be as many as six spherical nucleoli; in certain other *Rhizopoda* the "Binnenkörper kann den von der Kernhülle umschlossenen Raum nahezu völlig ausfüllen." In the *Heliozoa* the nucleoli are much as in the preceding group. In the *Radiolaria* (for which Bütschli follows some of the observations of R. Hertwig, '79) there is usually a number of rather large nucleoli, frequently containing vacuoles. The nucleus of *Thalassicola* "enthält einen ansehnlichen, strangförmigen und unregelmässig verästelten Nucleolus, dessen Masse nicht ganz homogen, sondern äusserlich feinkörnig ist"; it later breaks into a number of segments. In *Acanthometra* the nucleolus is at first spherical, while later "Aus dem Nucleolus-Pol, welcher der Einstülpungsstelle der Kernmembran zugewendet ist, bildet sich eine helle homogene Masse aus, welche den dunkleren Haupttheil des Nucleolus wie eine Kappe bedeckt oder auch wie eine Vertiefung desselben eingesenkt erscheint. Der Nucleolus erscheint demnach jetzt von zwei verschiedenen Substanzen zusammengesetzt." In many *Flagellata* a nucleo-

lus is absent, in others there is a single one, sometimes with a vacuole; in the *Choanoflagellata* there is always one large, spherical nucleolus, in the *Cystoflagellata* several of various sizes; and in the *Dinoflagellata* there may be several small nucleoli, which are sharply localized from the chromatin, but show the fine reticulation of the latter element. In the *Ciliata* and *Suctoria* there are nucleoli of varying size and number in the macronucleus, but none in the micronucleus.

Chun ('80) finds in the egg of all *Ctenophora* a single large nucleolus, very rarely two.

Engelmann ('80) figures the nucleoli of certain ciliated cells of various invertebrates as each surrounded by a clear space, the outer boundary of which is marked on optical cross-section by a circle of granules.

Flemming ('80) concludes in regard to the nature of the nucleolus: "Dass die Nucleolen überhaupt keinerlei morphologischen Antheil an der Kernvermehrung nehmen"; and "Dass die Dinge, die wir Nucleolen nennen, vielleicht gar keine morphologisch wichtige Theile des Kerns sein mögen, sondern nur Ablagerungen von Substanzen, welche für den Stoffwechsel im Kern verbraucht und wieder neugebildet werden; sie würden damit gewiss physiologisch wichtige Theile des Kerns bleiben, — was ohnehin durch ihr fast allgemeines Vorkommen bewahrt wird, — aber doch keine eigentlich organischen, d. h. morphologisch-wesentlichen Kernbestandtheile."

O. Hertwig ('80) found in the eggs of *Chaetognatha* numerous small nucleoli.

Shäfer ('80), ovum of *Gallus:* there is a single nucleolus, which in young germinal vesicles consists of a homogeneous matrix which stains slightly with haematoxylin, and a number of coarse granules which stain deeply; in larger ova the nucleolus is homogeneous throughout and stains deeply. The threads radiating from the periphery of the nucleolus may be either artefacts or may be regarded as extrusions of the homogeneous substance of the nucleolus. Ovum of *Lepus:* in younger nuclei the nucleolus has the same general structure as in the fowl, though it is more irregular in form. In some larger ova the nucleolus "is represented by a number (a dozen

or so) of globules of varying size which appear to lie loose within the germinal vesicle. An intravesicular network is sometimes present, and serves to unite the granules of the macula. . . . It is possible that the homogeneous matrix above described may represent the remains of such a network, the filaments of which have shrunk up into a mass on contact with the hardening reagent" (picric acid and alcohol).

Trinchese ('80, according to Platner, '86) found in the germinal vesicle of *Amphorina coerulea* a "macchia germinativa laterale *o* accessoria," and a "macchia germinativa principale," the latter being about seven times the size of the former.

*1881.*

Balbiani ('81) investigated the salivary gland cells of the *Chironomus* larva. There are here "Deux gros nucléoles irréguliers, larges de 0.03 à 0.04 mm., bosselés à leur surface, et formés d'une substance réfringente granuleuse, creusée d'un plus ou moins grand nombre de vacuoles isolées ou confluentes. Il arrive assez souvent que les deux nucléoles se confondent par une partie plus étroite qui les réunit comme une sorte de pont; d'autres fois enfin, ils se fusionnent plus ou moins intimement en un seul nucléole, dont le diamètre est le double de celui des nucléoles isolés." The ends of the chromatin filament are apposed against the nucleolus; and the latter differs both chemically and morphologically from this "cordon nucléaire."

Giard ('81) observed in the egg of a *Spionid* during life a single central nucleolus. A certain time before completed maturation an "élément cellulaire" appears in the nucleus, which is a little smaller than the latter, and encloses in its center a small "noyau": "D'abord fort éloigne du nucléole, il s'en approche progressivement et vient s'appliquer à sa surface, où il s'aplatit et prend la forme d'une double calotte. En s'appliquant de plus en plus contre le nucléole, il perd son noyau et finit par se réduire à une double membrane qui entoure le nucléole," . . . and finally its substance fuses with that of the nucleolus.

Hubrecht ('81), egg of *Proneomenia:* "in all the different stages of development of the ovum the germinal spot is double:

a larger and a smaller sphere may be distinguished, which, however, are not connected in any way whatever . . . but perfectly free and independent of each other."

Mark ('81) finds that during the maturation of the egg of *Limax campestris* the male as well as the female pronucleus may contain as many as fifty or sixty "pronucleoli," which disappear before the copulation of the two pronuclei. In an undetermined species of *Limax* he "observed in both female and male pronuclei a single nucleolus of much greater size and more deeply stained than the other nucleoli."

Pfitzner ('81) finds that the "Kernsubstanz" is contained in the reticulum and the nucleoli; the latter lie within the meshes of the former, and their rôle is problematical. "Während des weiteren Verlaufes der Karyokinese verschwinden sie, werden anscheinend allmälig aufgezehrt, ohne direkt mit dem Gerüst in Verbindung getreten zu sein."

Retzius ('81, cited by Van Bambeke, '85): the nucleoli are simple local accumulations of the chromatin, derived from the nuclear reticulum.

*1882.*

Blochmann ('82) observed in the egg of *Neritina* one large nucleolus containing a vacuole. Preceding the pole body production, the nuclear membrane vanishes, and the nucleolus at first retains its original size, then breaks up into several equal-sized fragments. "Dass die Elemente der Kernplatte aus Theilstücken des Nucleolus entstehen, kann bei unserem Objekt keinem Zweifel unterliegen, da ich alle Uebergangszustände vom unversehrten Nucleolus bis zur ausgebildeten Kernplatte beobachtet habe." After the two pole bodies have divided off, the remaining chromosomes in the female pronucleus fuse together to form a deeply staining, spherical body, which resembles the original nucleolus.

Flemming in his classical work ('82) gives the following definition of nucleoli: "Substanzportionen im Kern von besonderer Beschaffenheit gegenüber dem Gerüst und dem Kernsaft, fast immer vom stärkeren Lichtbrechungsvermögen als beide, mit

glatter Fläche in ihrem Umfang abgesetzt, stets von abgerundeter Oberflächenform, meist in den Gerüstbalken suspendirt, in manchen Fällen ausserhalb desselben gelagert." A membrane is absent around all nucleoli. He (erroneously) attributes the discovery of the nucleolus to the botanist Schleiden. Flemming holds it probable that with the possible exception of spermatozoa one or more nucleoli occur in every nucleus, of which it is therefore an important organ (in this conclusion he departs from the views expressed in his previous contribution, '80). "Die Zahl ist bei Thierzellen selten über 8 (mit Ausnahme der Kerne meroblastischer Eier), bei den meisten Arten von Thierzellen durchschnittlich 3–5. . . . Es ist der häufigste Fall, dass einer der Nucleolen an Grösse besonders vorwiegt," this being then the "Hauptnucleolus," the others "Nebennucleoli." In the "Hauptnucleolus" of the egg of *Lepus* two parts are distinguishable, but he leaves it undecided whether "die Unterscheidung von Haupt- und Nebennucleolen eine durchgehende Geltung beanspruchen kann." This investigator notes further: "Die absolute Grösse der Nucleolen steht bei den meisten Zellenarten in annähernder Proportion zur Grösse der Kerne selbst." The nucleolar vacuoles are filled with fluid. In regard to the apparent clear spaces around nucleoli, we read "dass dieses Phänomen nichts anderes ist als ein Reflex, bedingt durch die rundliche Fläche und stärkere Lichtbrechung des Nucleolus." He did not find amoeboid changes of form, but concedes that they may occur. The true nucleolar substance differs from the chromatin. The nucleoli are "specifische Produkte des Kernstoffwechsels und zugleich auch specifische Formtheile des Kerns . . . so kann man die Nucleolen ganz wohl Organe des Kerns oder der Zelle nennen." They appear to be "besondere Reproductions- und Ansammlungsstellen des Chromatins. . . . Entweder ist also in den Nucleolen noch ein anderweitiges Substrat vorhanden, in welchem das Chromatin verarbeitet wird und mit dem es in ihren durchlagert liegt, oder . . . die Substanz der Nucleolen mag zwar in sich homogen sein, ist aber dann nicht identisch mit Chromatin resp. Nuclein, sondern eine chemische Modification, Vorstufe oder Doppelverbindung derselben."

Graff ('82) figures in the eggs of *Proporus*, *Plagiostoma*, and *Vorticeros* a single nucleolus containing vacuoles.

Nussbaum ('82) studied the nuclei of gland cells (stomach nucosa of various *Vertebrata*, epidermis glands of *Argulus*). "Es liess sich im Allgemeinen feststellen, dass während des ungestörten Ablaufs der Secretion die mononucleolären Kerne vorherrschten, dass nach längerem Hunger die multinucleolären Kerne an Zahl vermehrt waren. . . . Ein Drüsenzellenpaar der Saugscheibe von *Argulus foliaceus* hatte am 12. Oktober mononucleoläre Kerne; am 18. Oktober zeigten sich viele Kernkörperchen im Kern; nach und nach ging die Granulierung der Zellen, die Strahlung verloren und die Kerne waren platte Ovoide mit mehreren glanzlosen Körperchen darin." From these observations Nussbaum concludes: "So wird man den Kern mit vielen Kernkörperchen als den Ausdruck einer Ruhepause der Kernfunctionen auffassen können, die entweder zum kräftigen Leben oder zum Tode überleitet."

Rauber ('82) figures the nucleoli in the ova of various vertebrates, and distinguishes the following kinds of nuclei, with regard to the mode of distribution of the "chromophile Substanz" (chromatin together with pyrenin): "globuläre," "trabekuläre, "filoïde," and "gemischte."

Seeliger ('82) finds that in *Clavelina* the nucleus of the loose mesoderm cell (from which the ovum is derived) becomes the nucleolus of the ovum, and its cytoplasm becomes its nucleus. In the germinal vesicle there is then one large nucleolus, in which nucleolini lie, and also (to judge from his figures) vacuoles.

Vejdovský ('82), egg cells of *Sternaspis scutata:* the young nucleus contains at first one small nucleolus, bounded by a membrane (though the latter structure would appear from his figures to be a clear space enveloping the nucleolus). "Beim fortschreitenden Wachstum des Keimbläschens vergrössert sich auch der Keimfleck, und zwar in der Weise, dass die ihn umgebende Membrane einseitig sich verdickt und schliesslich auf dem runden sich in Pikrokarmin stark färbenden Keimfleck als ein glänzendes, gelbliches Bückelchen erscheint." The nucleolus disappears in the ripe egg.

*1883.*

Balbiani ('83) renewed his observations on the egg of *Geophilus longicornis*, making several emendations. In very young eggs there are two or numerous nucleoli, in larger eggs only one large one, containing one or several vacuoles. In his previous paper referred to, he assumed that the double tubular structure in these eggs served for the purpose of an intraovular circulation; but in the present paper he offers another explanation: that the double tubular structure later develops into a knotted cord, the distal portion of which then divides into irregular fragments, which become scattered through the yolk; and then each of these fragments, with the exception of one which becomes the "noyau vitellin," differentiates into cytoplasm, nuclear and nucleolar substance, and then represents a cell of the follicular epithelium.

Van Bemmelin ('83) states of the eggs of *Brachiopoda:* "Sie haben meist zwei Kernkörperchen, die enganliegend und stark lichtbrechend sind. Ausser diesen nimmt man oft noch mehrere lichtbrechende Kügelchen in dem gefärbten Inhalte der Eikerne wahr. Von Boraxkarmin werden sowohl diese Körperchen als die Nucleoli stark gefärbt." (Certain of his figures show one of the nucleoli imbedded in another.)

Van Beneden ('83), ovum of *Ascaris megalocephala:* there is a single "corpuscle germinatif," which contains all the chromatin of the nucleus, and is contained within a special portion of the nucleus termed the "prothyalosome"; from one to three "pseudonucléoles" also occur in the nucleus, but they play no important part in the maturation of the egg.

Fol ('83a), egg of *Ciona intestinalis:* there is here one large, very refractive nucleolus containing a number of vacuoles which he believes are artefacts, since they cannot be found in the living egg, though their appearance after the action of reagents would show that the substance of the nucleolus is chemically not homogeneous. The nucleolus consists of a more refractive cortical substance, and of a less refractive, clearer medullary portion; in the latter, the vacuoles are produced. Fol maintains that the follicle cells arise by budding from the

egg nucleus : "Ce nucléole a une tendance bien évidente à se placer dans le voisinage immédiat des noyaux folliculaires en voie de formation. Le fait n'est pas constant, mais il est trop fréquent"; he did not actually observe that the nucleolus gives off a part of its substance to the follicle cell, but supposes this to be the case.

Fol, in a second paper ('83b) of the same year, finds that in *Ciona* during the "production endogène" of the follicular cells a segment (diverticulum) of the egg nucleus breaks off, while the (then peripherally situated) nucleolus gives a part of its substance into this diverticulum, and the nucleolus then wanders back to another portion of the nucleus. "Chez *Ascidia mammillata*, le bourgeonnement de l'enveloppe a lieu simultanément en une foule de points, et il est tout ou moins admissible que la substance de la tache germinative dispersée à la formation de ces bourgeons."

Gruber ('83) describes in *Actinosphaerium* the growth of a supposed nucleolus and its division during mitosis into two equatorial plates; though his figures would show that he mistook true chromatin masses for a nucleolus.

Jensen ('83) studied the ovum of *Cucumaria;* there are from fifteen to thirty nucleoli flattened against the nuclear membrane, and containing vacuoles. As shown by treatment with acetic or picrosulphuric acid, the outer layer of the nucleolus seems to be a continuation of the nuclear membrane, so that the inner, less refractive portion of the nucleolus appears to be situated in a depression of the outer surface of the nuclear membrane.

La Valette St. George ('83, quoted after Platner, '86) found in the egg of an *Isopod* one nucleolus which is at first homogeneous, later granular, and which may enclose a vacuole and show amoeboid movements. In other cases there are either several smaller vacuoles or one or two larger ones.

Leydig ('83), from comparative studies, concludes that the nucleoli "sind Theile des Kernnetzes," and that each of them is enclosed in a small, clear cavity of the nucleus. "Die Nucleoli können als eine Vielzahl von Körnchen erscheinen, die unter sich gleichwerthig sind. . . . Nicht selten lässt sich bei genauem Zusehen in der Menge kleiner und unter sich gleicher

Kernkörper ein grösserer Nucleolus . . . auffinden (Epithel des Eierstocks von *Aglia tau*). . . . Wahrhaft riesige Kernkörper kommen zu Stande, wenn viele Nucleoli zu einem einzigen Körper zusammenfliessen. . . . Prüfen wir Herkommen und Beschaffenheit der Kernkörper näher, so ist bezüglich der kleineren Nucleoli leicht festzustellen, dass sie aus Verdichtungen oder Knotenpunkten des Kernfadennetzes den Ursprung nehmen. Daher schon im frischen Zustande solche Kernkörperchen einen zackigen Saum haben, auch durch Spitzen und Striche sich verbinden, die bis zum Rande des Kernes gehen. Aber selbst die grösseren Nucleoli . . . erweisen sich als Umbildungen von Partien der Kernfäden." In the ganglion cells of the brain of *Limax* and *Arion* the nucleoli are jagged in outline, with long fibers. In the cells of the salivary gland of *Nepa* they are three or four in number, bent and elongated in form. Those of the corresponding gland in *Naucoris* have often the shape of a half ring, or may be lobular or band shaped, with cross striation. In the salivary gland of *Chironomus plumosus* there is usually a single nucleolus, spherical, lobular or tubular, its radiating cavity filled with a homogeneous, refractive substance ; its wall contains vacuoles, "und starke Linsen lassen deutlich werden, dass der ganze Kernkörper eben wieder die Struktur eines Schwammgebildes besitzt." Besides the nucleoli there are in these cells several looped or contorted bodies, one of which is always in connection with the nucleolus, and all of which evince a cross striation, the nature of which is as follows : "Mit Tauchlinsen unterscheiden wir abwechselnd je eine dunkle und helle Querlinie und sehen die erstere, welche leicht gekerbt ist, zusammengesetzt aus einzelnen kleinen Stückchen, vergleichbar den Elementen einer Muskelscheibe. Die feinen Abtheilungslinien der den Querstrich bildenden Stückchen erstrecken sich ferner durch die helle Zwischenzone, so dass dadurch auch eine Art von zärtesten Längslinien zum Ausdruck kommen kann." He believes that these cross-striated structures "durch Umbildung des den Kern durchziehenden Maschenwerkes entstanden sind." In young larvae these structures are not seen immediately in life, but "nach und nach, während das Thier noch lebt, tauchen die querstreifigen

Bildungen auf. . . . Man darf wohl annehmen, dass die fraglichen Gebilde, bevor sie dem Auge sichtbar werden, schon dagewesen sind und nur erst jetzt sich abheben, weil die Lichtbrechungsverhältnisse sich geändert haben." Similar cross-striated bodies were noticed in the cells of the Malpighian vessels of *Chironomus*. In the ovarial egg of *Libella* Leydig found one nucleolus, which consisted of a mass of granules grouped around a central cavity, these granules being connected together by fine threads ; "der lebende Nucleolus zeigt ferner langsam ablaufende Gestaltsveränderungen, wobei sich nach und nach einzelne Klümpchen mehr oder weniger absondern."

Ogata ('83) investigated the pancreas cells of man, which had been treated with various poisons and with the induction current, then fixed in aqueous solution of corrosive sublimate, and with osmic acid. One to more than eight nucleoli may be present: "Die einen färben sich wie die Kernmembrane tief mit Haematoxylin. . . . Die anderen oder vielmehr das andere, denn es ist in der Regel nur eins, färbt sich nicht mit Haematoxylin, sondern mit Eosin. . . . Manchmal hat es einen ganz feinen blauen Saum, als habe es selbst wieder eine Membran. Es ist viel grösser als die anderen Kernkörperchen, und das Feld, in dem es liegt, ist durch eine stärkere Linie von dem übrigen Kern getrennt. . . . Man wird es am unbefangensten wegen seiner Färbung als Plasmosoma von den übrigen die Kernfärbung annehmenden Karyosomen des Kerns unterscheiden." Sometimes several smaller plasmosomata are also present. Close to the nucleus is a body he terms "Nebenkern," which stains as the plasmosoma, but is much larger, and is apposed to the surface of the nucleus like a hat ; its substance is homogeneous, refractive, enclosing small cavities in which minute spherules occur, the latter having a resemblance to zymogen granules. The "Nebenkern" is produced by a plasmosoma which has wandered out of the nucleus, and there becomes the nucleus of a new cell. (This process is called "Zellneuerung.")

Pfitzner ('83) found in the resting nuclei of the ectodermal cells of *Hydra* usually one central, spherical nucleolus. Its substance is not identical with the chromatin in the resting stage of the nucleus, but becomes metamorphosed into the latter

substance during the following mitosis ; wherefore he suggests the term "Prochromatin" for nucleolar substance. In the prophase of the mitosis only one nucleolus is present in the nucleus, while in the "Rückkehr der Tochterkerne zum Ruhestadium waren dagegen stets mehrere vorhanden. In einem gewissen Stadium, wo die Nucleolenbildung beginnt, ist eine ganze Anzahl vorhanden ; jemehr sich der Tochterkern dem Ruhestadium nähert, desto mehr vermindert sich die Zahl unter gleichzeitiger bedeutender Grössenzunahme der übriggebliebenen, bis für das ausgesprochenste Ruhestadium das Vorhandensein eines einzigen grossen central gelegenen Nucleolus geradezu typisch wird," and this he concludes to be a process of fusion. The nucleolus plays only a passive rôle in mitosis, "nämlich die eines aufgespeicherten Nahrungsmaterials zur Neubildung von Chromatin."

Rein ('83) studied the eggs of *Lepus* and *Cavia*. In each there is one large nucleolus which disappears during maturation and is succeeded by several smaller ones, which have the same consistency as the first, and at the time of their first appearance occupy a central position in the nucleus. "So weit ich den Vorgang am Säugethiere verfolgen konnte, machte mir derselbe eher den Eindruck eines successiven Zerfalles des ursprünglichen Keimflecks in immer kleinere Stückchen, welche schliesslich in der Substanz des Keimbläschens verschwinden."

Roule's ('83) conclusions are, in the main, confirmatory of Fol's ('83) observations in regard to endogenous cell formation. In the egg of *Ciona* there is one large and two or three smaller nucleoli, the latter being "formés pendant l'évolution des cellules endotheliales en cellules ovulaires." In eggs a little larger these "nucléoles adventifs" become more numerous (five to six), and certain of them show a limiting membrane. Later still some of these adventive nucleoles are found in the yolk, where each becomes surrounded by a clear zone ; these he considers at this stage to be the nuclei of endogenetically formed cells (follicular cells), the clear zone around each representing its cytoplasm.

Schauinsland ('83) noticed in the egg of *Distomum* a single large nucleolus.

A. Schneider ('83) studied *Klossia*, one of the *Coccidia*. One or several nucleoli are present, "formant un ensemble souvent très complexe que j'appelerai le *corps nucléolaire*." Sometimes the largest nucleolus is enveloped on one side by a number of secondary, much smaller ones ("nucléolites"), which are portions loosened from the inner substance of the large nucleolus, from which they break out through a "canal micropylaire" (such a canal was not observed in life, and on only a single fixed preparation; *cf.* his Fig. 7). "Corrélativement à la multiplication du corps nucléolaire, le nucléole principal diminue de volume. . . . Touts les petits nucléoles qu'on observe dans le corps nucléolaire me paraissent descendre aussi sûrement du nucléole primitif ou ancêtre que les jeunes d'une espèce de leurs parents. Les nucléolites, une fois produits, grossissent et, d'homogènes qu'ils étaient d'abord, peuvent offrir à leur tour la différenciation d'une couche corticale et d'une zone centrale et faire office de producteurs nouveaux . . . j'ai de bonnes raisons de penser qu'à ce moment tous les nucléolites produits sont de taille sensiblement égale et qu'ils paraissent tous homogènes. . . . Je n'ai pas vu ce que deviennent ces fragments du nucléole, quelque soin que j'aie mis à scruter leur destinées. Je suppose que l'enveloppe du noyau se rompt, que les nucléolites mis en liberté gagnent par des mouvements propres la zone superficielle de la masse granuleuse pour s'y diviser activement. . . . Si ma hypothèse était fondée, le corps nucléolaire mis en liberté dans le plasma du kyste représenterait en réalité les débris de la fortune d'un noyau; ce serait le noyau lui-même, segmenté, morcelé, et le nom employé, celui de nucléoles, serait complètement impropre."

Weismann ('83), ova of *Hydromedusae:* in all the genera studied there is always a single large nucleolus, which sometimes contains one or several vacuoles.

*1884.*

Ayers ('84) germinal vesicle of *Oecanthus niveus:* in smaller eggs a single nucleolus, in larger ones several; these nucleoli he considers as "nodules of nuclear filaments."

Carnoy ('84) distinguishes three kinds of nucleoli: (1) "nucléoles nucléiniens," which are parts of the chromatin network; (2) "nucléoles-noyaux," which contain all the elements of a normal nucleus (namely, a membrane, chromatic filament, and nucleolar substance), while the substance in the remainder of the nucleus is allied to cytoplasm; such nucleoli occur in *Gregarines*, large *Radiolaria* and *Rhizopoda*, *Spirogyra*, the asci of lichens, testicle cells of *Littobius*, and eggs of *Pleurobrachia*, *Ascidia*, and *Nephthys;* (3) "nucléoles plasmatiques," which contain no chromatin, but consist of a plastin network in which an albuminous enchylema is imbedded.

Frommann ('84) studied fresh ganglion cells from the anterior horn of the medulla of the ox; their nucleolus shows "eine Zusammensetzung aus feinen und derberen Körnchen und aus sehr kurzen Fäden, mitunter auch einen netzförmigen Bau mit theils ganz engen, theils etwas weiteren Maschen." In the ganglion cells (of the ganglion Gasseri) of the rat, the nucleolus is usually homogeneous, as are those of the sympathetic ganglion cells of *Bufo*.

R. Hertwig ('84), *Actinosphaerium:* in the resting nucleus there is one central nucleolus which consists of deeply staining nuclein and faintly staining paranuclein. The nucleolus is rarely spherical; when so, it consists mainly of nuclein, except for a small portion of paranuclein superimposed on the margin. In other cases the larger nuclein portion is of a curved dumb-bell shape, and "gleichzeitig bildet das Paranuclein ein schwach gekrümmtes Stäbchen, dessen Krümmung zur Krümmung der Nucleinmasse senkrecht gestellt ist." The connecting portion of the dumb-bell may disappear, "so dass sich zwei Nucleoli bilden, welche von einander durch ein queres Stäbchen Paranuclein getrennt werden. . . . Hiermit beginnen die plurinucleolären Kerne, wie sie für gewöhnlich bei Actinosphaerium beobachtet werden." In most nuclei there lies a mass of from six to twenty nucleoli, which are smaller as they become more numerous: "Hier ist es sehr schwer festzustellen, was aus dem Paranuclein geworden ist, und . . . bin ich zu dem Resultat gekommen, dass es als ein Korn im Centrum des Haufens von Kernkörperchen ist, dass es mit einem Fortsatz an jedes

derselben herantritt und alle somit unter einander zu einer Rosette vereinigt. . . . Die staubförmigen Nucleoli sind ursprünglich vorhanden, erst allmählich vereinigen sie sich zu grösseren Stücken, bis endlich nur ein einziger Nucleolus und Paranucleolus gegeben ist; dann tritt die Theilung ein." In the resting nucleus all the chromatin is contained in the larger nucleolus.

Jijima ('84) found that there are one or several nucleoli in the ripe eggs of Triclad *Turbellaria*, but none in younger germinal vesicles.

Korschelt ('84), following Balbiani ('81) and Leydig ('83), investigated the interesting structures in the cells of the salivary gland of *Chironomus*. The form and number of the nucleoli is mainly such as was described by Balbiani, "meist aber sind sie ausgehöhlt und von der Form einer mit sehr dickem Boden versehenen Schale. . . . Die Convexität der Schale richtet sich immer nach der zunächst gelegenen Aussenfläche des Kernes. . . . Der Kernkörper besteht aus einer feinkörnigen Masse, in welcher Vacuolen auftreten. . . . Von den Vacuolen fliessen oft einander benachbarte zu einer grösseren zusammen." The cross-striated structures described by Balbiani are not to be seen in the fresh nucleus, but, as noted by Leydig, first appear after the nucleus has remained under the microscope for some time; thus they may be possibly products of coagulation. "Dass sie sich, wie dies Balbiani zeichnet, mit ihren fransenartig gebildeten Enden an die sog. Kernmembranen anheften oder dass (nach Leydig) Anheftungsfäden von ihrer Oberfläche zur Umgrenzung des Kernes hingingen, habe ich allerdings nie bemerken können. . . . Ich muss nach meinen Befunden . . . sagen, dass die "Querstreifung" der Bänder auf einer Faltung ihrer Oberfläche beruht und dass eine Zusammensetzung aus verschiedenartigen Schichten nicht vorhanden ist." Further, Korschelt did not observe the enveloping membrane of these structures, described by Balbiani, though he corroborates the observation of this author that the end of the band gradually fuses into the mass of the nucleolus. From experiments on starving larvae, he concludes: "Es scheint demnach das eigentliche Chromatin nicht die ganze Masse der

Bänder auszumachen, sondern nur einen Bestandtheil derselben zu bilden, der bei mangelhafter Ernährung der Gewebe zuerst schwindet."

Lang ('84) remarks of the egg cells of Polyclad *Turbellaria:* "Das Kernkörperchen oder der Keimfleck ist stets als ein kugliger, relativ sehr grosser, intensiv gefärbter Körper zu unterscheiden."

Vejdovský ('84) noticed a single nucleolus in the eggs of *Oligochaeta.*

Wielowiejski ('84) studied the egg cells of various *Arthropoda.* In the *Araneina* and *Acarina* the larger nucleolus contains a single large or several smaller vacuoles, though no pulsating or amoeboid movements were noticed (in opposition to the observations of Balbiani). In *Drassus* and *Lycosa* there is a small mass of granules in place of a germinal spot; in *Oniscus,* a single large nucleolus; in *Astacus,* numerous peripheral ones; and in *Musca,* a large, irregularly spherical one. (He notes that the germinal vesicle differs from all other nuclei in that its contents do not stain at all, or only faintly, with acetic acid methylen-green solution.)

Will ('84) studied in life the eggs of *Bufo* and *Rana.* Larger and smaller nucleoli may be distinguished; the latter increase somewhat in size, but never attain the dimensions of the preceding. Those nucleoli, then, which lie close to the nuclear membrane cause small protuberances ("Knospen") of this membrane, each such bud next breaks off from the nucleus, and, still enclosing a nucleolus within itself, wanders towards the periphery of the cell, and there becomes a "Dotterkern," the disintegration of which furnishes the yolk granules.

*1885.*

Van Bambeke ('85) reviews the opinions of the following writers in regard to the nature of nucleoli: Flemming ('82), Strasburger, Pfitzner ('81), Retzius ('81), Leydig ('83), Balbiani ('81), Korschelt ('84), R. Hertwig ('84), Van Beneden ('83), Frommann ('84), Carnoy ('84), Brass, Wielowiejski ('84), and Rabl ('84). Nucleoli are rarely absent, and hence they must be regarded as an essential element of the nucleus. "Le

mode d'origine des nucléoles généralement admis explique le rapport de ces éléments avec la charpente nucléaire. . . . Flemming est dans le vrai en disant que si les nucléoles sont généralement suspendus au *reticulum*, ils ne sont pas en continuité de substance avec ce dernier, mais constituent des éléments spéciaux. . . . Nous croyons devoir rapprocher du nucléole principal la formation récemment désignée par Ed. Van Beneden, sous le nom de *corpuscule germinatif*, et plusieurs de celles appelées par Carnoy *nucléoles-noyaux*." The nucleoli are probably reservoirs for masses of chromatin.

Van Beneden and Julin ('85) found, in contradiction to Roule, that in the ovum there is only a single large "corpuscule germinatif" in *Clavelina*, and neither smaller nucleoli nor any migration of nucleoli into the cytoplasm.

Bütschli ('85), *Ceratium tripos:* most of the nucleoli of the individuals examined contained no nucleoli; only occasionally are one or two present, and then these evince a honey-combed ("wabige") structure. In many *Flagellata* there is no trace of a nucleolus.

Carnoy ('85) amplifies his observations of the preceding year, in which he had distinguished the following four types of nucleoli: (1) "nucléoles nucléiniens"; (2) "nucléoles plasmatiques"; (3) "nucléoles mixtes" ("qui sont constitués par la réunion des deux espèces précédentes en un corps unique"); (4) "nucléoles-noyaux." Types 1, 3, and 4 are closely related, and all are sharply demarcated from type 2. The "nucléoles plasmatiques" are plasmatic, albuminoid accumulations, and not chromatin material in reserve (in opposition to the views of Heuser, Guignard, and Pfitzner): "Nous préférons dire qu'ils concourent avec les autres éléments plasmatiques du noyau à l'élaboration du fuseau, dont les filaments constituants sont formés d'une substance, ou de diverses substances, présentant beaucoup d'analogie avec la plastine." The "nucléole nucléinien" may be composed of amorphous masses or of a skein of chromatin (the latter is the case in the testicle cells of *Chilopoda*, ova of *Pleurobrachia* and *Cymbulia*): "Le nucléole central de beaucoup de cellules ganglionnaires est de nature nucléinienne et présente souvent

la même constitution filoïde"; and similar nucleoli occur in the *Protista* and in various cells of the *Arthropoda*. The "nucléole-noyau" of the eggs of *Cymbulia* and *Lithobius* has a fine external membrane and a convoluted chromatin filament. In the amitotic division of the capsular ovarial cells of *Gryllotalpa* the nucleolus (formed of a central portion of chromatin and a peripheral layer of plastin) divides first so that each daughter-nucleus receives one nucleolus. But in the amitosis of the intestinal cells of *Aphrophora* the "nucléoles plasmatiques" do not divide; and in the testicle cells ("métrocytes") of *Scolopendra* there is also a "nucléole plasmatique," and at the commencement of the mitosis the "nucléole se liquéfie pour enrichir le caryoplasma," and is not to be found later. The amitotic division of the fat cells of *Geotrupes* is introduced by a division of the "nucléole-noyau."

Frenzel ('85) studied the cells of the mid-gut in insects at various stages of development. *Bombyx dispar*, larva: one large nucleolus containing a vacuole, in which lies a small spherical "Nucleollolus." *Tachina*, larva: here is one large nucleolus, "mit kurzen zackigen Ausläufern. In seinem Innern umschliesst er fast stets wenigstens einen, in der Regel aber mehrere, etwa 6 bis 12, kugelige oder matt aussehende Gebilde, welche nicht gerade den Eindruck von festeren Körpern, sondern vielmehr von Vakuolen machen." In cylinder and gland cells of various insect larvae the nucleus is filled with a homogeneous fluid, "in welche sowohl echte Nukleolen, wie auch nucleolenartige Körper ('Keimflecken' oder 'Nukleolide') einerseits und andererseits zahlreiche verschieden angeordnete sehr klein aber stets gleich grosse Körnchen eingelagert sind, die hier 'Kerngranula' oder '-granulationen' heissen mögen."

Leydig ('85) noticed in ganglion cells of *Astacus* a large, spherical, granular nucleolus, in which is a large cavity; this nucleolar cavity stands in communication with that of the nucleus itself. We read further: "Die Körper im Kern, die man Nukleoli nennt, sind Bildungen verschiedener Art"; some arise out of the nodal points of the nuclear network, others out of the "Kernplasma."

Rabl ('85) studied mitoses in cells of the larva of *Salamandra*, and found that in the prophases of mitosis the nucleoli gradually vanish and take part in the production of the chromatin threads. In the unripe germinal vesicle of *Proteus*, on the inner surface of its membrane, "sieht man in unregelmässigen Abständen von einander kugelige, stark glänzende, wie Oeltropfen aussehende Körperchen," which he assumes are neither nucleoli nor masses of true chromatin.

Will ('85) studied the ovogenesis of *Notonecta* and *Nepa*. The young "Ooblast" contains one nucleolus bounded by a membrane and surrounded by smaller "Chromatinballen"; subsequently the latter bodies fuse together and form a closed ring around the nucleolus. The nuclear division of the oöblast is an amitotic one, and is preceded by a division of its nucleolus; in each daughter-nucleus, then, the divided half of the primitive nucleolus breaks up into fragments, which become distributed through the nuclear sap, and the daughter-nucleus produces a new nucleolus without the aid of these particles. When the ovum proper is ripe, the nucleolus finally disappears.

*1886.*

Van Bambeke ('86) found that in the germinal vesicles of *Arachnida*, *Isopoda*, *Hymenoptera*, and *Meconema*, the nucleoli and the chromatin do not stain with methylen green (corroborating Wielowiejski) though they stain with carmine and haematoxylin; "Rien ne s'oppose, me semble-t-il, à ce que l'on considère le corpuscule germinatif comme étant équivalent à l'ensemble de la charpente chromatique des noyaux ordinaires [somatiques]." He concludes that there is no proof of the identity of the true nucleoli of the somatic cells with the germinal spots of egg cells. Two stages in the formation of the nucleolus may be distinguished in the ova of various Arachnids (*Lycosa*, *Amaurobius*, *Argyronecta*, *Tegenaria*, *Attus*, *Theridium*, *Epeira*, *Zilla*, *Phalangium*): (1) there is a single large nucleolus (sometimes accompanied by smaller accessory ones), in which at first a few vacuoles arise, which later fuse to produce a single voluminous vacuole; and (2) the nucleolus becomes replaced by a mass of fine granules. In the ovarial egg of *Amaurobius*

*ferox* the nucleolus consists of (1) a peripheral, less deeply staining portion; and (2) of a more deeply staining and more highly refractive central portion, in which one large and several smaller vacuoles lie: "Chose remarquable dans la vacuole centrale se voyait, à l'état frais, un granule foncé, doué d'un mouvement très vif"; in this germinal vesicle a small, finely granular nucleolus is also present. Amoeboid movements of the germinal spot of *Periplaneta* were noticed. In the egg of *Zilla* there are from one to three homogeneous, spherical nucleoli, as also a large "tache principale"; the latter is composed of two or three different substances, somewhat as in *Amaurobius*.

Carnoy ('86), egg of *Spiroptera strumosa:* there is one large, central "nucléole nucléinien," sometimes also one or two small "nucléoles plasmatiques"; the former nucleolus is the only part of the nucleus which stains deeply with methyl green; it is bounded by a fine membrane, and contains eight "bâtonnets" (chromosomes), so that it is comparable to a "nucléole-noyau." *Nematode* from the stomach of *Scyllium canicula:* in the "œufs très jeunes . . . le filament nucléinien y est assez puissant, il paraît continu. . . . Nous n'avons pu voir s'il se scindait d'abord en tronçons; nous croyons plutôt qu'il se localise par le retrait de ses anses, pour constituer un nucléole nucléinien pelotonné. Ainsi naît la tache de Wagner. Elle est toujours simple; elle se colore peu par le vert de méthyle"; no "nucléoles plasmatiques" are present in this nucleus. In the egg of *Filaroides mustelarum* one or two "nucléoles plasmatiques" occur; but in that of *Ascaris lumbricoides* such nucleoli are usually absent, and the chromatic filament extends through the whole nucleus. In *Ascaris* sp. (from the dog) there is one "nucléole plasmatique" in young eggs.

Heathcote ('86) noticed in the egg of *Julus* one nucleolus with vacuoles; it disappears before the production of the pole bodies.

Knappe ('86), ovarian ova of *Bufo:* The nucleoli show amoeboid movements in life, and these movements probably lead to the dissolution of the nucleoli, by causing the latter to first break into fragments, these fragments afterwards dissolving in the nuclear sap.

Pfitzner ('86a) distinguishes in the nucleus: "Das Achromatin, eine geformte färbbare Substanz, das Chromatin (mit der Unterart der Nucleolensubstanz, des Prochromatins) und eine geformte nicht färbbare Substanz, das Parachromatin." In a second paper ('86b) he studied *Opalina:* here are several nucleoli flattened against the nuclear membrane; "bei der Kinese verschwinden sie allmählich, aber später als bei anderen Objekten bisweilen sind sie noch bis zur Metakinese vorhanden." Though they are occasionally found at the poles of the spindle they take no part in the formation of the chromatin elements, and in the daughter-nuclei reappear at a distance from the latter elements. For denoting the substance of the nucleoli he substitutes for his earlier term "Prochromatin" the term "Pseudochromatin," since "das Chromatin und die Nucleolensubstanz wohl nichts Anderes mit einander gemeinsam haben, als die untergeordnete Eigenschaft, sich bei den meisten Färbemethoden gleicherweise stark zu färben."

Platner ('86) investigated the ovogenesis of *Arion* and *Helix*. In *Arion* there appears first in the "primitives Ei" a small, completely spherical nucleolus, to which he limits the name "Nucleolus"; "weiterhin enthält das Keimbläschen den eigentlichen Keimfleck. Dieses ist zu Beginn seines Auftretens meist rundlich mit hervorspringenden Erhabenheiten, als sei er durch Contraktion eines Knäuels entstanden. Zuweilen erscheint er auch mehr ringförmig oder ganz unregelmässig. Immer aber verdichtet er sich bald zu einem völlig runden homogenen Element, welches Kernfarbstoffe begierig aufnimmt und den Nucleolus bedeutend an Ausdehnung übertrifft." (His figures show the two to be in close contact.) A number of clear vacuoles begin to appear in the "Keimfleck": "Sie sind rund und von verschiedener Grösse . . . und scheinen nur dazu zu dienen, weitere Veränderungen einzuleiten. Sie verschwinden nämlich alsbald wieder, und in dem stetig an Grösse zunehmenden Keimfleck scheidet sich mit wachsender Deutlichkeit eine heller gefärbte und eine dunklere Partie. Letztere, dem "corpuscle germinative" van Benedens entsprechend, ist von geringer Ausdehnung, rundlich oder länglich oval und liegt excentrisch in der von runden Contouren

begrenzten hellen Substanz, die demnach auf dem Querschnitte halbmondförmig erscheint. Sie dürfte dem von van Beneden als "prothyalosome" bezeichneten Gebilde entsprechen. Es sei mir daher gestattet, sie Hyalosoma zu benennen. In völlig entwickelten Eiern ist dieses Element nahezu völlig farblos und erscheint aus feinen Körnchen zusammengesetzt. Die gefärbte Partie des Keimflecks tritt dadurch um so schärfer hervor, man kann sie im Anschluss an van Beneden Keimkörperchen nennen." The nucleolus of the ripe egg "liegt excentrisch und besteht wieder aus dem runden zart granulirten Hyalosoma, sowie in dem peripher in demselben gelagerten Keimkörperchen, welches sich stark färbt und keine weitere Differenzierung erkennen lässt. Dem hellen Hyalosoma meist dicht anliegend findet sich der intensiv sich färbende Nucleolus oder der kleinere Keimfleck." Platner considers that by the last division of the ovocyte the "Nebenkern" disappears and becomes a constituent of the nucleus. "Bei Ausbildung der Furchungsspindel konnte ich mit Sicherheit constatiren, dass die Spindelfasern aus der unfärbbaren Substanz des Eikerns hervorgingen. Diese ist bei sich entwickelnden Eiern im Keimfleck enthalten, in welchem sie sich bald als Hyalosoma differenzirt." In *Helix* the "primitive Eier . . . entbehren des schönen grossen Nucleolus. . . . Daher enthält ihre definitive Form auch nur einen Keimfleck, welcher weiterhin dieselben Veränderungen zeigte wie bei *Arion*." It may be noted in conclusion that in the spermatogonium of *Arion* the nucleolus appears in the nucleus at the same time that the "Nebenkern" appears in the cytoplasm.

Schauinsland ('86) found one or two large nucleoli in the egg of *Bothriocephalus rugosus*.

Stuhlmann ('86) investigated the early stages of the ovum in a large number of species, more particularly of the *Arthropoda*. *Carabus memoralis:* there are numerous "chromatin" granules in the young eggs, which increase in number and size; later a granular nucleolus appears: "Es ist schwer zu entscheiden, ob der Haufe von chromatischen Körnern zu einem grossen Ballen zusammenschmilzt, oder ob sich einer, wohl das ursprünglich central gelegene, zum Nucleolus ausbildet oder endlich ob

letzterer eine ganz neue Bildung ist. . . . Wenn aber schon Dotter ausgeschieden ist, hat der Nucleolus fast stets eine Form, die aufs Täuschendste einer Eichel gleicht. . . . Wir sehen an dem Nucleolus einen helleren, völlig homogenen Theil und einen dunkler gefärbten, welcher fein granulirt ist und wie mit einer Menge von winzigen Vacuolen durchsetzt erscheint. Dieser dunklere Theil umgreift wie die Cupola einer Eichel den helleren Theil. Um die Formähnlichkeit ganz zu vollenden, sitzen häufig auf der Kuppe der homogenen Hälfte noch einige dunkle Körnchen. . . . Auf einem Aequatorialschnitt sieht man nun, dass der dunklere Theil eine Zone um den helleren Theil bildet." This enormous nucleolus measures 67$\mu$; it disappears when the nucleus wanders to the periphery of the egg. *Carabus auratus* and *Pterostichus elatus:* one spherical nucleolus, containing a few small vacuoles, and its size increases with that of the nucleus; later it assumes a peripheral position, "und in seiner Nähe treten mehr oder weniger kleine Chromatinkugeln auf, während der Nucleolus selbst kleiner zu werden scheint"; the nucleolus disappears, then the small "Kugeln" unite to form a larger spherule, and finally the latter also vanishes. In the egg of *Dytiscus marginalis* there are no true nucleoli, only irregular masses of chromatin. Egg of *Silpha:* one granular nucleolus, which increases in size up to a certain point, and later, when vacuoles arise in it, a number of small spherules become apparent outside of the nucleolus: "Ob dieselben aus dem Nucleolus stammen oder ob sie als Paranucleolen des Kerngerüstes aufzufassen sind, weiss ich nicht," though he does not think that they are products of the nucleolus; the nucleolus, as well as a portion of the nucleus, disappears later. *Necrophorus vespillo:* several non-homogeneous germinal spots, later a single nucleolus, which finally vanishes. Eggs of *Geotrupes* and *Cetonia:* several small, spherical or elongated nucleoli, which occupy a central position in the nucleus, and increase in number and size; "Dieselben liegen in concentrischer Anordnung um einen homogenen Kern, der etwas dunkler als die Kerngrundsubstanz gefärbt ist." *Lina populi:* at first there is one large and one small nucleolus; in this stage "sind im ganzen Keimbläschen

mit Ausnahme der Randzone ganz feine klare Bläschen vertheilt, welche ich jedoch als Kunstprodukte ansehen möchte"; later there lies in one part of the nucleus a group of minute nucleoli; then a portion of the nucleus breaks off and wanders into the cytoplasm, while the remaining portion of the nucleus retains one small nucleolus; and lastly, when the nucleus becomes amoeboid in shape, it contains one large vacuolated nucleolus, "sowie mehrere kleinere chromatische Körper." *Lycus aurora:* at first there is no nucleolus, later a large and a small one (both granular); when the nucleus wanders to the periphery of the egg it retains one of the nucleoli, which subsequently disappears at the same time as the nucleus does. *Periplaneta orientalis:* at first there is no nucleolus, "derselbe bildet sich erst allmählich heraus. . . . Wir sehen ausser dem etwas körnigen Nucleolus eine Anzahl kleinerer stark färbbarer Kügelchen, die wohl als Bestandtheile des Kerngerüstes, als Paranucleolen aufzufassen sind." *Gryllotalpa vulgaris:* in the immature egg "ein eigentlicher Keimfleck ist nicht vorhanden; vielmehr liegen in der Kerngrundsubstanz zerstreut Chromatinpartikel von 4$\mu$ Durchmesser bis zu unmessbarer Feinheit"; when the nucleus has assumed a peripheral position a large nucleolus is produced in it, "wohl durch Verschmelzung mehrerer kleinerer." *Locusta viridissima:* in maturer ova a large but lightly staining nucleolus, "von dem aus ein Kernnetz seinen Ursprung nimmt." *Pieris brassicae:* one large, homogeneous germinal spot, which later acquires vacuoles and divides into three parts. *Sphinx ligustris:* in the immature germinal vesicle lies a large, excentric nucleolus, containing vacuoles; "ausser letzterem finden sich noch einige wenige Paranucleolen"; at the time when the nuclear fragments break off, the nucleolus becomes paler and then vanishes. *Zygaena filipendulae:* at first no nucleolus is present, later there is a larger one with vacuoles, as well as a smaller one, "der sich wohl von dem grossen abgelöst zu haben scheint"; subsequently both disappear. *Musca vomitoria:* there is at first in the germinal vesicle a single, large, excentric nucleolus, but later appear in it "eine Anzahl von Paranucleolen und ein Nucleolus, . . . von welchen letzterer aus einem Häufchen von

kleinen, gefärbten Kügelchen besteht." In the egg of *Anabolia* there is one large nucleolus, but in those of *Vespa germanica* and *V. media* apparently no true nucleoli are present. There is a large granular nucleolus in the larger germinal vesicles of *Bombus terristris*. *Trogus lutorius:* there is one large, irregularly shaped nucleolus and two smaller ones; all these finally disappear, and their place is taken by smaller granules. *Banchus fulvipes:* at first no nucleolus is present, later one or three large nucleoli appear, but all of them vanish subsequently. In the egg nucleus of *Pimpla* sp. only a number of small granules are to be found, and at a later period still smaller granules. *Anomalon circumflexum:* in the youngest germinal vesicles no nucleolus is to be found, in older ones there is a single large one; this has nothing to do in the formation of the "Dotterkerne," and disappears when the nucleus does. There is one spherical germinal spot in *Ophion ventricosum*, but not in *O. luteum*. *Ephialtes liturater:* in the smaller nuclei a considerable number of "chromatic" bodies occur, while in the older ones there is a single large nucleolus. *Ambyteles castigator:* one large nucleolus, in older ova also several smaller ones. *Epeira diadematа:* here is one large spherical nucleolus, which later becomes jagged in outline and evinces vacuoles, which may unite to produce a single larger vacuole: "In seltenen Fällen kann man einen Zerfall des Nucleolus in mehrere kleinere sehen, was jedoch wohl eine pathologische Erscheinung sein dürfte." *Glomeris marginata:* one large, spherical or angular nucleolus, and later also a smaller one: "Höchst wahrscheinlich stammt dieser von dem grossen Nucleolus ab"; the smaller nucleolus disappears subsequently. In the egg of *Peripatus edwardsii* one nucleolus forms itself gradually, and vacuoles begin to appear in it. In *Amaroecium rubicundum* a single large nucleolus is present; while in *Clavelina lepadiformis* the nucleolus is probably formed out of the central chromatin masses. From these numerous observations Stuhlmann draws the conclusion: "Aus Allem schien mir hervorzugehen, dass das Schwinden des Nucleolus nicht zum Wesen der Eireifung gehört, besonders weil ich ihn bisweilen (so bei *Silpha*) so lange verfolgen konnte, als noch ein Rest des Keimbläschens im Ei sichtbar war."

Vigelius ('86) finds in the egg of *Bugula* one large nucleolus, containing vacuoles.

Will ('86) studied the maturation of the egg of *Colymbetes.* "Dem Kernkörperchen oder Nucleolus . . . kann nach meinen Untersuchungen keinerlei morphologische Bedeutung zukommen. Was wir Kernkörperchen nennen, ist nach meiner Auffassung nichts als ein besonders grosses Stück Chromatinsubstanz. So können wir es verstehen, dass bald eines, bald mehreres, bald gar keine vorhanden sind."

*1887.*

Boveri ('87): in the ovum of *Ascaris megalocephala bivalens* there are no true nucleoli when the tetrads are formed. In the variety *univalens* there is usually one "achromatisches kugeliges Körperchen. Von dem "Prothyalosoma," das an den van Beneden'schen Eiern den Keimfleck [Vierergruppe] umgiebt und welches im weiteren Verlauf bei ihm eine so grosse Rolle spielt, habe ich weder auf diesem Stadium, noch später die geringste Spur wahrgenommen."

Eisig ('87) remarks in regard to the egg of *Capitellids:* "Der ursprünglich rundliche, jederzeit durch Dichtigkeit und hohes Tinctionsvermögen auffallende Keimfleck erleidet im Laufe seines Wachsthums offenbar Theilungen; denn man findet ihn in späteren Stadien mit ein oder zwei verschiedengradig abgeschnürten Kuppen besetzt; ausserdem trifft man schon frühe mehrere Pseudonucleoli, welche offenbar Produkte des Hauptnucleolus darstellen, in dem Keimbläschen zerstreut." He notes, further, that in the maturing ovum the nucleolus does not increase in size in equal proportion to the size of the nucleus. (To judge from his figures, the nucleoli are not homogeneous.)

Fraipont ('87) found in the germinal vesicle of *Polygordius* several nucleoli of unequal size.

Henking ('87) studied the eggs of *Phalangids.* In the ovarial egg a sickle-shaped body lies at one pole of the nucleolus: "es scheint, als wenn in ihm und dem Keimfleck die Chromatinsubstanz des Keimbläschens sich koncentrirt hätte." In the nearly ripe egg there is one large nucleolus, which is not homo-

geneous, and a number of smaller globules, these latter staining as the former, and some of them containing vacuoles: "sie stellen einerseits eine Zusammenballung der bisher ganz unregelmässigen, im Keimbläschen vertheilten Chromatinsubstanz dar, rühren andererseits aber wohl vom Keimfleck her." These bodies have all disappeared in the ripe egg.

Hubrecht ('87) noticed only a single nucleolus in the egg of *Cerebratulus* sp.; as to the egg of *Pelagonemertes*, he figures one nucleus containing one large and several smaller nucleoli, and another nucleus with only numerous small nucleoli.

Kosinski ('87, '93, mentioned by Lavdowsky, '94): within the nucleolus of cancerous cells there is sometimes a vacuole, and within the latter a small body which Kosinski considers may correspond to Carnoy's "nucléoles-noyaux"; such nucleoli have the faculty of division, and of wandering through the nuclear membrane into the cytoplasm.

Lukjanow ('87a), stomach epithelium of *Amphibians:* in the cytoplasm of the cylinder epithelium are structures of various form ("Nebenkerne"), which stain in general like the nucleoli. In some of the nuclei of the deep layer of gland cells each nucleolus is joined with a karyosome.

Lukjanow ('87b) distinguishes three kinds of nucleoli in muscle cells of *Vertebrates:* (1) "Plasmosomen"; (2) "Karyosomen"; (3) "Kernkörperchen von gemischtem Charakter." The first stains deeply red (eosin), the second blue-violet (haematoxylin), while the third stains a mixed color with these two stains (when used together). He remarks also: "dass in manchen Kernen die Kernkörperchen gänzlich fehlen, in anderen entweder nur eine Kategorie derselben, oder mehrere zugleich vertreten sind. . . . Zuweilen liegt das Kernkörperchen sogar ganz ausserhalb des Kernes."

Nussbaum ('87) found in smaller eggs of *Hydra* a single large nucleolus, while in larger ova several are present. "In frischem Zustande sieht man in den allerersten Stadien neben den Keimflecken noch eine blasse Kugel, die im Gegensatz zu den Nucleolen des Keimbläschens keine Farbstoffe in sich aufnimmt."

O. Schultze ('87) studied the maturation of the egg in *Rana* and *Triton.* In the unripe germinal vesicle there are larger

nucleoli near the nuclear membrane, and smaller ones at the center of the nucleus: "Dass sie sich durch Theilung vermehren, kann keinem Zweifel unterliegen, denn nicht nur sind dieselben in ganz jungen Eiern grösser und weniger zahlreich, . . . sondern die grösseren Keimkörperchen weisen durch Einschnürung und Zerklüftung auf eine Vermehrung durch Theilung hin." He does not consider that such daughter-nucleoli are again capable of division, but that the process is rather a "Lösungsphänomen." All the nucleoli are homogeneous, but vacuoles are produced in them by ½ per cent normal salt solution. In larger ova a considerable number of nucleoli lie peripherally, and there is also a central group of them; and, still later, the peripheral nucleoli commence to stain less intensely, and the greater number are centrally situated. The nucleus of the maturing egg consists of "Membran, Kernsaft und Keimkörperchen," a chromatin network being absent; and the microsomes of the chromosomes are formed from the smallest, most centrally placed nucleoli.

*1888.*

Böhm ('88) found in the egg cell of a 5 cm. long *Ammocoetes* of *Petromyzon* a homogeneous nucleolus, "an dem sich sehr oft eine kleine Vacuole zeigt, welche mit einer feinen Strasse bis an die Oberfläche des Fleckes [nucleolus] reicht." At the animal pole of the nucleus lies a disc-shaped mass ("Deckel"): "räthselhaft ist die Bedeutung des Deckels." (Compare the extranuclear structure found by Lukjanow, '88.)

Boveri ('88): in the female pronucleus is neither a prothyalosoma nor a hyalosoma, such as were described by Van Beneden ('83); the hyalosoma is probably "ein durch Schrumpfung entstandenes Artefakt." Just before copulation "zeigen sich die ersten Spuren achromatischer Kernkörperchen als ganz kleine Körnchen, die . . . stets . . . in nächster Nachbarschaft der chromatischen Elemente sich finden, . . . so dass die Vermutung nahe gelegt wird, dass sie sich aus diesen absondern."

Fiedler ('88) studied the egg development of *Spongilla:* one large homogeneous nucleolus is present in the germinal vesicle. In the nuclear division (which is intermediate between the

mitotic and the amitotic) "der gesammte sonstige — übrigens spärliche — Chromatininhalt des Kernes vereinigt sich . . . mit dem Kernkörperchen zu einem kugeligen Gebilde, und erst dieses zerfällt dann durch allmähliche Zerschnürung in zwei kleinere, unter sich gleich grosse Kernkörperchen, welche an die beiden Pole des Kernbläschens rücken."

Graff ('88) found in the egg of *Spinther* either a mass of granules or a single nucleolus; the nucleolus may be either granular or contain a large vacuole.

R. Hertwig ('88): in nuclei occur "chromatische" nucleoli, and "das unter gewöhnlichen Verhältnissen nicht färbende Paranuclein, welches zumeist rundliche Körper, die Paranucleoli, bildet. Die Paranucleoli können entweder die einzigen Kernkörperchen im Kern sein (gewöhnliche Gewebszellen, reifes Ei und Furchungszellen) oder sie finden sich neben den chromatischen Nucleoli, unter Umständen auch als Einschlüsse derselben (Keimbläschen der unreifen Eier, Kerne von Actinosphaerien und anderen Protozoen) vor. . . . Zweifelhaft wird es dagegen gelassen, ob auch der Substanz des achromatischen Gerüstes . . . nicht . . . vielleicht auch Paranuclein [ist], welches sich durch seine Anordnung von den Paranucleoli unterscheidet." The centrosomes are probably derived from the paranucleoli, and the paranuclein is "die befruchtende Substanz" (these views have subsequently ('96) been retracted).

Kultschitzky ('88) found in the youngest eggs of *Ascaris marginata* one "Kernkörperchen," which afterwards "in zwei Stückchen zerfällt, deren eines sich intensiv mit Karmin färbt und alle Eigenschaften des Chromatins bewahrt, das andere sich in die blasser gefärbte gewöhnliche Kernkörperchen verwandelt"; the latter he terms the true "Kernkörperchen," which from this stage on gradually decreases in size, and finally disappears.

Leydig ('88) gives the results of numerous comparative investigations on germinal vesicles; most of these observations were made on the living egg, fixing reagents having been little employed. *Nephelis vulgaris:* here there is one nucleolus, which sometimes has a long process, "in dessen Nähe kleine rundliche Ballen von gleicher Art, wie er selber ist, liegen, so

dass man die Entstehung der letzteren durch Abschnürung von dem Fortsatz sich denken darf." *Argulus foliaceus:* in young eggs there is one large nucleolus with clear spaces in it, showing that the nucleolus "aus Theilen besteht, die allmählich von einander weichen, so dass man alsdann in anderen Thieren anstatt eines Keimflecks eine ganze Anzahl kleinerer vor sich hat"; these nucleoli are often jagged in contour; by treatment with chromo-acetic acid "bekommen die Keimflecke eine Querzeichnung, so dass sie wie aus Querstücken zusammengesetzt erscheinen." *Tetragnatha:* one large nucleolus with dark contours, and several smaller pale, granular ones, which gradually disappear during the maturation of the egg. *Lycosa:* "Ein einziger, grösserer Keimfleck zeigt sich . . . und dieser bietet das Bild eines Knäuels dar." *Theridium:* the large "Hauptkeimfleck hat die Beschaffenheit eines stattlichen, aus scharf geränderten kleinen Körpern zusammengesetzten Ballens. Von ihm nun weg zieht sich ein Strang solcher Körperchen oder Theilstücke über die Grenze des Keimbläschens hinaus in den Dotter hinein. In einzelnen Eiern, deren grosser Keimfleck das Bild gewundener und geknäuelter Fäden giebt, können die kleinen Theilstücke zusammenhängend oder in bereits abgelösten Gruppen abermals in den Dotter sich erstrecken. Ja ich glaube an dem lebenden Ei verfolgt zu haben, wie Theile der geknäuelten Fäden sich zu einzelnen Ballen zusammenschoben und in den Dotter vordrangen"; there are present also one or several pale "Nebenkeimflecke." *Phalangium:* the young ovum has one large nucleolus containing vacuoles; "Wiederholt habe ich beobachtet, dass ein solcher Keimfleck — das lebende Ei mit Mundspeichel befeuchtet — unter dem Mikroskop allmählich verblasste und zuletzt für das Auge völlig verschwand." *Lithobius:* there may be one granular nucleolus, or numerous nucleoli, each with a granular core: "Wieder eine andere Form ist die, dass die amöbenartigen Gebilde in ihrem Innern einen hellen, kernartigen Fleck mit centralem Pünktchen zeigen und am Rande feinstrahlig sind"; in other germinal vesicles there may be present numerous small nucleoli, either irregularly grouped or arranged in "kurze, goldrollenähnliche Säulchen . . .; ein andermal stösst man auf

längere fadige Aufreihungen, deren Stränge zu Schlingen gebogen oder geknickt sind." In these ova two kinds of nucleoli may occur, namely, numbers of the small ones just described, and a large one with dark contours, which has a central vacuolar, granular portion, and is peripherally homogeneous; but nucleoli also occur which are intermediate between these two kinds. *Geophilus electricus:* here are numerous small, pale nucleoli and a large one, which has a finely granulated core, and an outer homogeneous layer, the latter portion consisting of concentric layers; further, he noticed the infundibular structure first found by Balbiani on the outer surface of the nucleus, though he remarks that it is especially apparent in eggs in which post-mortem changes have commenced (!), and concludes: "Wir haben es sonach bezüglich des Trichters mit einer Ausbuchtung jenes Hohlraumes oder Lichtung zu thun, welche von der Höhlung um das Keimbläschen herum in den Dotter dringt." The basis of this infundibulum empties into a space around the nucleus, and not into the nucleus itself (as opposed to Balbiani's observations); Leydig also thinks that particles of finely divided nucleoli penetrate separately out of pores which are present in the nuclear membrane, and that these particles, arrived in the cytoplasm, fuse together to form a large "Ballen." *Stenobothrus:* in the ova of the proximal portion of the egg tube there are either numerous small nucleoli or a dense mass of very fine granules; in riper germinal vesicles they are much larger and resemble somewhat the nucleoli in the salivary glands of *Chironomus;* masses of nucleolar substance wander out of the nucleus into the cytoplasm. In *Pemphigus bursarius* there is one compound nucleolus, with fine radiating processes; and in *Meloë violaceus* there are numerous nucleoli, each of which has the structure of the single one of the preceding species. *Gasterosteus aculeatus:* in the month of May there are numerous germinal spots, sometimes densely grouped, sometimes arranged in rows; the gradual thickening of the nuclear membrane takes place at the cost of nucleolar substance. *Triton taeniatus:* the germinal vesicle at the end of October contains numerous nucleoli of unequal size, many of which are arranged in columns; the peripheral ones probably

wander into the cytoplasm. *Salamandra maculosa*, larvae: the "Urei" has a single large nucleolus. *Bufo cinereus*, larvae of several months: concludes "dass die Keimflecke, wenn noch winzig klein, aus den Knotenpunkten des Spongioplasmas entstanden sind, und nachdem sie eine gewisse Grösse erreicht, die Form und Sonderung einer Amöbe besitzen. Dieselben stellen sich jetzt dar wie hüllenlose, kleine Zellen, an denen wir einen homogenen körnigen Körper, der feinzackig oder selbst in feine Strahlen ausgezogen ist, unterscheiden und im Innern einen lichten, kernähnlichen Fleck, in dem sich noch ein Körperchen abzeichnet"; numbers of such nucleoli may later fuse together, "unter Vermittelung ihrer Zackenspitzen." *Rana esculenta:* in the smallest ova there is only a single large nucleolus, with a vacuolar central portion and peripheral radiating strands; in larger eggs there are a number of smaller nucleoli, each of which has the same structure as the primitive one; Leydig believes that nucleoli wander out of the nucleus, since he found a granular mass on the outer surface of the latter. The ova of *Sus scrofa*, *Myoxus nitela*, and *Talpa europea* contain each a single nucleolus.

Lukjanow ('88) investigated the stomach mucosa of *Salamandra.* There are several, usually club-shaped nucleoli ("Nucleoli claviformes"), the smaller, often funnel-shaped, end of which is in contact with the nuclear membrane. He concludes "dass die kolbenähnliche Form des Nucleolus . . . auf eine Vorbereitung zur Inhaltsentleerung hinweist. Der Kolben entleert seinen Inhalt etwa ebenso, wie die Becherzelle ihren Schleim entleert"; and he supports this conclusion with the observation that a mass is often found on the outer surface of the nuclear membrane which stains like the nucleolus.

Nagel ('88) studied the human egg. The "Primordial-Ei" has a single nucleolus; those which contain no nucleoli he believes do not develop further. In the ripe egg amoeboid motions were noticed in life (studied in liquor folliculi).

Sanfelice ('88) terms the nucleolus of the spermatoblast "nucleus," and the nucleus, "cell." What he calls the nucleus then divides karyokinetically (but that this process is a division of the nucleolus may be deduced from his figures 60 and 62).

Scharff ('88) studied the maturation of the eggs of various *Teleosts.* In the smallest ova examined (.011 mm.) there are numerous peripheral nucleoli, and a few which are central in position. In larger eggs (.03 mm.) "the nucleoli show an inclination to gather still more towards the periphery of the nucleus . . . one or more of the nucleoli become larger than the others, and in their interior refractive specks are visible which have sometimes been described as endonucleoli." In still larger ova (.08 mm.) "in some cases the big nucleoli disappear almost completely, leaving an unstained part around them." In *Conger* he "noticed a small nucleolus being constricted off from a larger one." He figures outside of the germinal vesicle of *Gadus* certain granules, and these he considers are emigrated nucleoli which are destined to become dissolved there, though he holds it possible that "some find their way to the surface of the egg to form the nuclei of the follicular epithelium"; in eggs which have attained the dimensions of .132 mm. the nucleoli become very irregular in shape. In the *Trigla* egg of .13 mm. the surface of the nucleolus is raised into small protuberances, most of which contain a nucleolus; these protuberances later break off and become the yolk spherules (in corroboration of Will, '84).

Schewiakoff ('88), *Euglypha:* the nucleolus gradually disappears in the prophasis of mitosis.

Steinhaus ('88), intestinal cells of *Salamandra:* karyosomes and plasmosomes are distinguished within the nucleoli, and are usually combined in pairs with one another. Plurinucleolar nucleoli are formed by continued divisions of a single nucleolus, "et les nouveaux nucléoles s'éloignent l'un de l'autre, probablement à l'aide de mouvements amoeboides ou d'autres qui leur sont propres." Plasmosomes when extruded into the cytoplasm increase greatly in size, though this increase is due to mere imbibition of some substance; each such extruded nucleolus, combining with a karyosome, develops into a new nucleus.

Vejdovský ('88) studied the maturation of the egg of *Rhynchelmis.* The embryonal genital cells contain no true nucleoli. The nucleolus does not stain when it first appears (in very young stages). Subsequently it is always excentric in position,

perfectly spherical, and consists of a central, homogeneous, deeply staining portion, and an outer unstaining envelope (judging from his Fig. 5, Tab. 3, I would consider this supposed envelope to be a vacuole in which the nucleolus lies). In the more advanced ovum this envelope has disappeared, and the nucleolus has increased in size, but is no longer homogeneous, since it contains a number of deeply staining granules. When "das Kernkörperchen die oben angedeutete Grösse [.013 mm.] erlangt hat, beginnt es sich einzuschnüren, was gewiss auf dessen Theilung hinweist"; he believes that this division is rapid, "dass es aber thatsächlich so geschieht, beweist die Thatsache, dass in den reiferen Eiern in der Regel zwei Kernkörperchen vorhanden sind. Das neu entstandene Kernkörperchen liegt anfänglich in der Nähe des älteren und ist etwas kleiner als dieses; später entfernt es sich mehr oder weniger und wächst zu der Grösse des ersteren heran." In the ripe egg two nucleoli are present, or there may be three or four, the latter two having been divided off from the former; each of these consists of an inner chromatic portion and an achromatic envelope; the latter is porous, and "man kann voraussetzen, dass durch die Poren die flüssige Nahrung in das Innere des Kernkörperchens eindringt." When this envelope has vanished, each nucleolus is formed of (1) a hyaline, homogeneous fluid, in which (2) a delicate network arises, the nodal points of which are represented by the previous granules of the nucleolus; "kurz und gut, die Kernkörperchen unserer Eier sind chromatische Kernfaden. . . . Die intensive Färbung sowohl der Knötchen als des Fadenwerkes erleichtert die Verfolgung des metamorphosirten Kernkörperchens, welches jetzt ganz und gar den Kernen des späteren Blastomeren gleichkommt." (The descriptions do not enable one to determine whether all the nucleoli become thus metamorphosed.)

Waldeyer in his "Referat" ('88) agrees with Klein "dass die Nucleolen nur stark verdickte Knotenpunkte des Netzwerkes der Gerüstfäden [chromatin], also mit den letzteren identisch seien. . . . Die Bedeutung aller dieser Dinge für das Zellenleben ist noch fast vollkommen dunkel."

*1889.*

Bergh ('89), *Urostyla:* the fragments of the macronucleus contain true nucleoli, while the micronuclei do not.

Brass ('89) states: "Für gewöhnlich erscheint jedes Kernkörperchen rund, sehr häufig kugelrund; es besteht entweder aus einer gleichartigen Masse oder es sind in derselben einige hellglänzende Körnchen ausgeschieden, oder aber es finden sich in ihm dichtere, weniger glänzende Körnchen. . . . Im Umkreis der Kernkörperchen ist vielfach ein heller Hof, der von feinen Körnchen kugelschalenartig umgeben wird. Der Hof wird als Kernkörperchenhof beschrieben; er ist in sehr vielen Fällen sichtbar zu machen."

Davidoff ('89) observed in the egg of *Distaplia* a single large, spherical nucleolus, consisting of a homogeneous mass in which a few granules are imbedded. These nucleoli increase in size as follows: "Sie werden grössere Partien des Reticulums in sich aufnehmen, sich mehr und mehr verdichten und demgemäss sich immer deutlicher und deutlicher färben." Subsequently, but antecedent to the production of the pole spindle, the nucleolus contracts, and its contour becomes irregular, often with regular branched processes: "Vielleicht, ja sogar wahrscheinlich, werden sie dadurch hervorgerufen, dass der Nucleolus Flüssigkeit ausscheidet"; and the central portion of the nucleolus becomes lighter in color. Next, first the lighter portion, then the whole nucleolus, becomes filled with fine granules ("Chromatosomen"). Then these chromatosomes collect and form in the center of the nucleolus a compact, granular body, in the middle of which is one especially large chromatosome, and the whole is surrounded by a membrane. And finally, other chromatosomes, not concerned in the formation of the central granular body, form a reticulum around it. Davidoff concludes "dass aus dem Nucleolus ein Kern mit Kernnetz, mit einem Nucleolus und Nucleolinus hervorgegangen ist. Wir können diesen Kern weder als Keimbläschen, noch als Nucleolus bezeichnen. Es ist eben ein neues Gebilde, dass wir einstweilen mit dem Namen Polkern belegen wollen"; out of this "Polkern" the first pole spindle is formed.

Fol ('89), ovarian egg of *Dentalium;* the nucleolus is at first absent, and single. In larger nuclei there are two apposed nucleoli (which disappear when the nuclear membrane has vanished). "Le nucléole présente d'abord deux parties distinctes, dont l'une, plus volumineuse et moins foncée, entoure l'autre un peu comme un bonnet posé sur la tête. La partie foncée est sphérique ; elle retient l'hématoxyline ou le carmin alunique avec une nuance rougeâtre ou vineuse. Sa texture est compacte. L'autre partie est formée des corpuscules plus clairs [vacuoles] et d'un réseau plus foncé ; elle prend les colorants que nous venons de nommer avec une teinte violacée tirant sur le bleu. . . . Lorsque l'ovule approche de l'époque où la vésicule germinative va se dissoudre, les deux nucléoles, au lieu de s'emboîter, sont simplement accolés, et le nucléole clair s'est accru beaucoup plus que l'autre."

Hermann ('89a) investigated the spermatogenesis of the mouse. The "Spermatoblastkern" (nucleus of a v. Ebner's cell) possesses one nucleolus, which is made up of two parts, "einen von Safranin sehr intensiv gefärbten, und einen ungefärbt bleibenden Bestandtheil. Letzterer tritt stets in Form einer einfachen Kugel auf, die chromatische Substanz aber besteht entweder aus zwei kleinen, leuchtend roth tingirten, an zwei gegenüberstehenden Polen der farblosen Kugel liegenden Kügelchen, oder das chromatische Element stellt eine einzige, in diesem Falle grössere Kugel dar, die dem ungefärbten Bestandtheile des Nucleolus sich innig anschmiegt. Im ersteren Falle erscheint dann das ganze Kernkörperchen als ein annähernd spindelförmiges Element, im anderen als eine Doppelkugel, und ist in beiden Fällen die Längsaxe des Nucleolus stets in dem grössten Durchmesser des Zellkerns eingestellt." The nucleoli of the spermatogonia are sometimes biscuit-shaped. Those of the spermatids are at first multiple in number, but later they unite to form a biscuit-shaped one. Still later, by the formation of the spermatozoön out of the spermatid, the two parts of this nucleolus wander apart, "dabei aber noch durch eine chromatische Brücke mit einander in Verbindung stehen." He observed in the follicle cells of the testicle of *Salamandra* "neben kleinen Nucleolen einen

grösseren, . . . der vollkommen die gleichen Strukturverhältnisse zeigt, wie sie oben von dem Kernkörperchen der Spermatoblastkerne der Maus beschrieben wurden und wie dies für den Frosch von Sanfelice angegeben wird."

Hermann ('89b), testicles of immature white mice : the nuclei of the follicle cells contain compound nucleoli, similar to those of the cells of v. Benda of *Salamandra.*

Korschelt ('89) made observations on the germinal spots of *Epeira, Dolomedes, Phalangium, Spinther,* and *Ciona.* In *Epeira* the nucleolus is at first a compact mass of granules "von stark lichtbrechenden Körnchen umlagert. . . . Ich will damit nicht sagen, dass eine direkte Aufnahme von [nutritiven] Körnchen stattfände, welche letzteren sich dann unmittelbar zum Keimfleck formirten, sondern möchte vielmehr glauben, dass die Substanz in flüssiger Gestalt aufgenommen und erst im Kern wieder geformt wird"; in later stages small vacuoles are frequently present in the nucleolus. In *Dolomedes* the nucleolus is at first homogeneous, it later contains vacuoles, and finally becomes simply a membrane surrounding a cavity. In *Spinther* there is a single large nucleolus with a vacuole. Korschelt draws the general conclusion : "Ich muss es nach meinen Erfahrungen, . . . als zweifelos hinstellen, dass eine Auflösung der Nucleolarsubstanz stattfindet. Die Erklärung dieser Erscheinung fand ich darin, dass die Nucleolarsubstanz in und vielleicht ausserhalb des Kerns zur Verwendung gebracht werden sollte."

Lukjanow ('89) describes the nucleoli ("plasmosomata") of the germinal vesicle and cleavage nuclei ; they disappear during mitosis.

Platner ('89a), Malpighian tubule cells of *Dytiscus,* fixation in Kleinenberg's fluid : there are one or several nucleoli, of irregular form, and around each one usually "ein hellerer Hof, welcher aussen von einer Anzahl grösserer unregelmässiger Chromatinbrocken eingefasst wird." The division of the nucleoli introduces the amitosis of the nucleus : "Der anfangs mehr runde Nucleolus zeigt eine Abplattung zur Scheibe, welche der umgebende Hof mitmacht. Zugleich tritt in der Richtung seiner kürzern Durchmesser eine Streifung an dem-

selben auf, als wenn er aus einer Anzahl nebeneinander liegender schmaler Elemente zusammengesetzt wäre. Weiterhin tritt eine Spaltung in der Richtung des längsten Durchmessers auf. . . . Die auf diese Weise entstehenden Tochterplatten zeigen an den einander zugewandten Seiten spitze Hervorragungen, an den abgekehrten Flächen dagegen mehr abgerundete Erhabenheiten. Beide besitzen wieder eine längsgestreifte Struktur, als seien sie aus parallelen Stäbchen zusammengefügt. . . . Den auseinanderweichenden Tochterplatten passt sich der helle, umgebende Hof an, der also in der Richtung dieser Bewegung sich verlängert."

Platner ('89b) contends, in opposition to the views of Ogata ('83) and others, that in the pancreas cells the nucleoli do not wander out of the nucleus.

Platner's ('89c) observations on the egg of *Aulastomum* shall be mentioned in the course of our observations on the egg of *Piscicola*. In accord with O. Schultze ('87) he finds in amphibian ova that the contents of the nucleus are composed only of "Kernsaft und Keimkörperchen," a portion of the latter forming the nuclear filament, the rest being extruded from the nucleus; the true chromatin loops were not seen by him.

Weismann and Ischikawa ('89) find in the ovarial winter ova of *Leptodora* one large nucleolus (rarely is a smaller one apposed to it), containing a large vacuole; it wanders out of the nucleus and becomes the "Nebenkern, Paranucleus," which ultimately disappears, and corresponds to the nucleus alone of the paracopulation cell of the other Daphnids. In nearly ripe ova of *Bythotrephes* "findet man . . . innerhalb des Keimbläschens und dem Nucleolus desselben ganz nahe einen scheibenförmigen Körper, der sich wie der Nucleolus färbt. Etwas später, wenn das Keimbläschen bereits an die Oberfläche des Eies gestiegen ist, liegt dieser Körper ausserhalb des Keimbläschens und ist in einen Protoplasmahof eingebettet"; then it rapidly disappears.

Wheeler ('89), ovarial follicle cells of *Blatta:* there is a "nucleolus of unusual structure. The latter consists of an irregular mass, not stainable in carmine or methyl green, and is regarded as plastin by Carnoy. . . . The mass of plastin encloses

a smaller mass of chromatin, or at least of a substance which does not differ in its reactions from the chromatin of the coiled filaments in the same nuclei." This nucleolus divides first in mitosis.

*1890.*

Auerbach ('90) distinguished two kinds of chromatin substance: "erythrophile," *i.e.*, substances staining with eosin, fuchsine, aurantia, carmine, picrocarmine; and "kyanophile," substances staining with methyl green, aniline blue, haematoxylin. The nuclear reticulum is not the fundamental portion of the nucleus, but the nucleoli are its important elements. He finds "dass in einer Grundsubstanz, die im frischen Zustande homophan, im gehärteten . . . höchstens feinkörnig erscheint, grössere, scharf begrenzte, isolirte, stärker lichtbrechende und stärker färbbare Körperchen, Nucleoli, von wechselnder, aber für die verschiedenen Zellarten und Thierspecies typischer Anzahl eingebettet sind"; thus in the *Batrachia* most of the nuclei contain numerous nucleoli, and when they are particularly abundant the greater number are peripheral in position. There are two kinds of "Kernkörperchen," those which stain blue (or green) and those which stain red (or yellow); both kinds occur in most nuclei. In the giant nuclei of the gland cells from the skin of *Urodelea* are found (1) numerous small cyanophilic nucleoli, and (2) from one to fifteen (usually two to five) much larger, erythrophilic nucleoli, which sometimes contain vacuoles. Embryonal nuclei contain only cyanophilic nucleoli, while in maturer nuclei erythrophilic nuclei become differentiated from the former. Thus in the blood corpuscles of frog larvae there is at first only one large nucleolus, which later differentiates into an inner erythrophilic and an outer cyanophilic portion. The peripheral layer next breaks up and divides into small cyanophilic nucleoli, while the central portion remains as a large erythrophilic nucleolus. Subsequently the smaller cyanophilic nucleoli ("Nebenkügelchen") may fuse together so as to produce six or eight larger cyanophilic nucleoli, each of which attains the size of the original "Stamm-Nucleolus"; at the conclusion of the larval period of the frog, the latter

nucleolus entirely disappears, becoming dissolved in the nuclear sap. "Die erythrophile Kernsubstanz ist übrigens dem Protoplasma des Zellleibes offenbar ähnlicher als die kyanophile."

Bürger ('90) made observations on the maturing ovum of various *Nemerteans*. *Carinella:* there is one large, spherical nucleolus. In the ripe egg of *Cerebratulus marginatus* "in der Regel kann man zwei umfangreiche Keimflecke konstatiren, welche aus einer schwärzlich-grünen körnigen Substanz zusammengesetzt sind, aber einen membranartig scharfen Kontour besitzen. Die beiden Keimflecke sind nicht von gleicher Grösse." In the immature germinal vesicle of *Drepanophorus:* "Dem wenig tingirten Binnenraum des Kernes durchflicht ein zartes Netzwerk feiner Fäserchen; peripher sind gröbere dunklere Körnchen angeordnet"; the ripe ovum of this Nemertean contains one finely granular, central nucleolus, in which are found "kuglige, noch intensiver gefärbte Körperchen." *Prosadenoporus janthinus:* constituting the inner portion of the wall of the genital ducts are seen numerous cells, "welche ganz wie in der Entwicklung im frühen Stadium stehen gebliebene Geschlechtsprodukte aussehen," and each of these cells has one large nucleolus; while in the ripe egg the "Keimbläschen ist ausgezeichnet durch eine Menge kugliger Bläschen von über 5$\mu$ Durchmesser mit scharf kontourirter und stark gefärbter Peripherie."

Eimer ('90, cited by Mann, '92), recalls his previous observations ('73, '78) in regard to the termination of nerve fibrils in the nucleolus; he mentions further that such radiating fibers are also to be found in the nucleolus of the egg cell, such fibers serving at first as paths for nourishment, and later becoming nerve fibrils.

Henking ('90), spermatogenesis of *Pyrrhocoris:* the single peripheral nucleolus of the first spermatocyte gradually becomes smaller in the prophase of division, and it is considered probable "dass er späterhin eine Einschnürung erfährt."

O. Hertwig ('90), *Ascaris megalocephala:* in the spermatocytes of the growth zone the nucleolus is usually flattened against the periphery of the nucleus, or it may be irregularly elongated, or in addition to it a "Nebennucleolus" may be also

present; from these differences in form he concludes that the nucleolus may be capable of amoeboid movements. Subsequently it wanders towards the center of the nucleus, becomes larger and more spherical. When the chromatin has assumed the characteristic radial distribution, before the first maturation division, the nucleolus passes again towards the periphery, and there becomes gradually smaller, partly by fragmentation, and so gradually disappears.

Holl ('90) found one spherical nucleolus in ova of the newly hatched chick: "Da das Kernkörperchen so auffallend verschieden vom Kernnetze und Kernsafte hinsichtlich des Verhaltens zur Farbe sich zeigt, so muss es wohl aus einem anderen Stoffe bestehen als jene. Auch bei *Salamandra*, *Rana*, und *Lacerta* fand ich das Kernkörperchen immer sich verschieden halten von den anderen Theilen des Kernes." The nucleolus is always situated excentrically at the upper pole of the nucleus. Towards the end of the spirem stage the nucleolus lies on the periphery of the chromatin, with which it stands in no close connection; it is no longer present in ova of 491$\mu$ diameter.

Kastschenko ('90) investigated the maturation of the ova of *Pristiurus*, *Scyllium*, and *Torpedo*: there are numerous nucleoli, which attain a diameter of 16$\mu$, and all disappear at the spirem stage (in the prophase of the first pole spindle). Each nucleolus contains a large unstaining globule (but in his Fig. 1, in several of the nucleoli, all of which had been stained with borax carmine, this globule is colored blue, while the peripheral portion of the nucleoli is red).

Masius ('90): in the ovum of *Asplanchna* the nucleolus forms the greater part of the nucleus. In *Lacinularia* it is at first as in the preceding genus, but at a later stage several much smaller nucleoli are found.

Mellissinos and Nicolaides ('90), pancreas cells of *Canis*: The "Nebenkern" is a plasmosome which has wandered out of the nucleus; this migration is caused by an injection of pilocarpin into the living gland.

Sheldon ('90) found one germinal spot in *Peripatus capensis*, which disappears when the nucleus reaches the periphery of the egg.

Smirnow ('90), sympathetic ganglion cells of *Rana* and *Bufo*: a "Kernkörperchenkreis" is figured around the nucleoli of some of the cells.

*1891.*

Brauer ('91) studied the maturation of the ovum of *Hydra*. As a rule in the smaller eggs there is a single large nucleolus which occupies an excentric position within the nucleus; in larger ova numerous small nucleoli arise, which gradually become grouped near the large one. "Die Anzahl [der kleinen] wechselt, was zum Theil darin seinen Grund zu haben scheint, dass der grosse — selten sind zwei grosse vorhanden — wahrscheinlich durch Aufnahme kleinerer wächst . . . zum Theil aber auch darin, dass in verschiedenen Keimbläschen die Masse der Nucleolen eine verschieden grosse ist, was mit der Ernährung zusammenhängen möchte. . . . Sehr oft lag in der Nähe des grossen Nucleolus eine etwa halb so grosse blasse Kugel . . . möglich wäre es, dass diese sich vom grossen Nucleolus abgespalten hat, und den achromatischen Theil derselben vorstellt." Just before the formation of the first pole spindle the large nucleolus breaks into fragments, which, together with the smaller nucleoli, wander towards the periphery of the nucleolus: "Ein Theil scheint im Keimbläschen selbst aufgelöst zu werden, ein Theil tritt unverändert nach dem Schwinden der Membran in das Eiprotoplasma über." Brauer contends that the nucleoli have no morphological significance in the maturation of the egg.

Cuénot ('91), ovarial egg of *Synapta inhaerens*: "la tache germinative primitive bourgeonne une quantité de petits nucléoles secondaires, qui errent dans le protoplasma clair de la vésicule germinative; presque toujours la tache a un aspect mamelonné par suite de la formation de ces nucléoles."

Davenport ('91) figures in the germinal vesicle of *Plumatella* a double nucleolus.

Macallum ('91), following Ogata ('83), distinguishes two kinds of nucleoli, namely, plasmosomata and karysomata. He finds the "Nebenkerne" of Nussbaum to be abnormal structures. In the pancreas cells of *Amphibia* an extrusion of plasmosomata occurs, but it is not a normal process, and the extruded portion

does not become a "Nebenkern" (in opposition to the views of Ogata). In the eggs of *Rana* and *Necturus* the chromatin is "principally collected in the form of nucleoli at the periphery," but it is also contained in certain threads in the nucleus. He concludes from the study of the reactions of the substances to the indigo-carmine stain: "the peripheral nucleoli generate a substance, therefore, which diffuses gradually through the nucleus, then into the cell protoplasm, the point in time of the latter occurrence corresponding with the formation of the yolk spherules. The mode of origin is through a process of deposition from the nucleus of a substance allied to chromatin in the cytoplasm. . . . I regard the yolk spherules as formed by the union of a derivative of the nuclear chromatin with a constituent of the cell protoplasm."

C. Schneider ('91) concludes that the true spherical nucleoli "ebenso wie die Klumpen [of the chromatic network] aus [achromatischen] Gerüst und Chromatin bestehen und die Unterschiede beider nur morphologischer Natur sind." In the testicle cells of *Astacus* the nucleoli are spherical, with "eine deutliche Membran an und durch welche genau wie bei der Kernmembran [achromatische] Gerüstfäden treten. . . . Der ganze Unterschied zwischen Nucleolus und Klumpen besteht also hier darin, dass um ersteren die Fasern zu einer Membran sich zusammenlegen . . . was man ringförmig am Rande des Nucleolus wahrnimmt, ist sicher nicht die optische Wiedergabe einer Membran, sondern durch das Brechungsvermögen der Wandung des Nucleolus veranlasst." The nucleolus in eggs of *Echinodermata* is homogeneous only in the final stages of its formation. Nucleoli are only metamorphosed portions of the true chromatin, and represent reserve masses of this substance: "die Zusammenballung kann nur eine Befreiung der chromatischen Substanz von ihrer Arbeitsleistung bedeuten."

Wolters ('91) studied the sporulation of *Monocystis:* in the youngest individuals there is one nucleolus, which "in seinem Innern sich stärker tingirende chromatische Kugeln führt." In larger individuals the nucleolus consists of eight spheres, "Diese Kugeln führen in ihrem Innern wieder Stäbchen und Körner." Just before the conjugation of two individuals this compound

nucleolus breaks into a number of nucleoli of various sizes. After the copulation and encysting the nucleoli fuse together and gradually disappear (but I am unable to determine from his description whether the substance of the chromosomes is derived from the nucleoli). Shortly after the nuclei themselves copulate, the nucleoli reappear in them. In *Clepsidrina blattarum* there is a single primitive nucleolus, formed as in the preceding species; later there are numerous smaller nucleoli, which have probably arisen by division from the primitive nucleolus.

*1892.*

Bannwarth ('92) figures a division of the nucleolus in leucocytes from the spleen of the cat.

Born ('92) finds that in the *Amphibian* egg, in opposition to the observations of O. Schultze ('87), the chromatic "Fadenknäuel" has no origin in the nucleoli, but is directly derived from the chromatin network of the "Urei."

Brauer ('92) made observations on the maturation and fecundation of the egg of *Branchipus*. Each germinal vesicle from the "Wachsthumszone" of the ovary has one large, slightly staining nucleolus, and near it a much smaller, deeply staining one. Each "Nährzelle," however, contains numerous nucleoli, and its nuclear sap also stains deeply. When the chromosomes are being produced, the larger nucleolus of the egg cell gradually ceases to stain, and it finally disappears. In the male pronucleus small nucleoli are present.

Frenzel ('92) noticed in *Carcinus moenas* and in a species of *Amphipod*, in the ferment cells and "Fettzellen" of the hepatopancreas, amitotic division of the nucleus, but no division of the nucleolus; "sondern dass vielmehr an geeigneter Stelle des Tochterkernes noch vor der Abschnürung desselben ein ganz neuer Nucleolus entstehe, der alle Charaktere des ersten besitzt"; in this nuclear division one of the daughter-nuclei retains the whole original nucleolus. In similar cells of *Idotea tricuspidata* he found the nuclear division to be as in the preceding species (but his Figures 8b, 10, and especially 11, would seem to represent stages of division of the nucleolus).

Häcker ('92a) studied the early development of *Aequorea forskalea:* in the ripe egg there is one spherical or kidney-shaped nucleolus, containing vacuoles. At the time of the first pole body mitosis the nucleolus does not accompany the nucleus, but remains behind in the cell, at the place previously occupied by the nucleus; and from this time on he applies to it the name "Metanucleolus." It is to be observed in one of the cleavage cells until about the 32-cell stage. "Zur Zeit wenn sich dann in der schwärmenden Blastula die Zellen des hinteren Poles . . . zu differenziren beginnen, kann man in einzelnen von ihnen neben dem chromatischen Fadenknäuel kleine nucleolenähnliche Körper beobachten, welche den nicht differenzirten Blastula-Elementen fehlen. Es wäre denkbar, dass man es hier mit den Abkömmlingen des Metanucleolus zu thun hat, ich vermag aber weder hierrüber, noch über das weitere Schicksal dieser Gebilde etwas bestimmtes zu sagen." Häcker assumes that what Metschnikoff ('86) supposed to be the "Sperm-nucleus" in *Mitrocoma* was in reality a Metanucleolus; and also that the "Paracopulationszelle," described by Weismann and Ishikawa ('89) in the winter egg of *Daphnia,* to have been also a nucleolus.

Häcker in a second paper ('92b) studied the maturation of the ovum of *Canthocamptus.* In the smallest eggs the nucleolus is large and contains vacuoles. Later it becomes differentiated into a lighter central portion and a denser peripheral part containing small vacuoles. At this stage the nucleolus presents a concavity facing the chromatic spirem. Then "aus dem Kernkörper tritt unter plötzlicher Verkleinerung desselben eine Masse aus, welche vermuthlich dem grossen, bis dahin in den meisten Kernkörpern kugligen Einschluss entspricht." The nucleolus apparently disappears when the first pole spindle is perfected.

Heidenhain ('92), cells of *Salamandra:* the nucleoli lie enclosed within the chromatin and linin network; he was unable to decide whether each nucleolus has a particular chromatin envelope. The nucleolus has no processes, and "nur die ihm auflagernde, von ihm selber stofflich differente Schicht ist mit dem Chromatin- und Lininfadengerüst kontinuierlich verbunden. . . . Mir ist es wenig wahrscheinlich, dass die Substanz

der Nukleolen etwas dem Chromatin ähnliches sei. Zwar sind sie durch einige Chromatinfarbstoffe stark färbbar, wie z. B. durch Safranan, allein auf eine andere Gruppe derartiger Farbstoffe reagieren sie nicht, hierher gehört das Methylgrün."

O. Hertwig ('92) in his recent text-book materially changes some of the views expressed in his previous papers. The true nucleoli consist of "Paranuclein" (Pyrenin), and he uses the term "Nuclein" for chromatin. "Nuclein und Paranuclein betrachte ich als die wesentlichen Substanzen des Kerns. . . . Beide scheinen mir in irgend welchen Beziehungen zu einander zu stehen." Further, he distinguishes "Keimflecke" from "echte Nucleolen." "Je nach dem Alter oder der Entwicklungsstufe einer Zelle kann der ruhende Kern . . . in der Zahl, Grösse und Beschaffenheit seiner 'Nucleolen' erhebliche Veränderungen erleiden."

Kostanecki ('92a) is preliminary to his '92b.

Kostanecki ('92b) studied mitoses "in sämtlichen embryonalen Zellen" of *Lepus*, *Cavia*, and *Equus*, with especial regard to the central spindle; I quote this paper here, since the "Centralspindelkörperchen" may have some relation to nucleoli. "Im Bereich dieser Centralspindel sieht man in diesem Stadium [Dyaster] in der Nähe der beiderseitigen Tochterfiguren der Chromosomen kleine Körperchen auftreten, die ich als "Centralspindelkörperchen" bezeichnet habe. Grösse und Zahl dieser Körperchen zeigen ganz beträchtliche Schwankungen. . . . Meist fand ich nun jederseits vier, fünf oder sechs grössere Körperchen, . . . daneben aber immer noch eine grössere Anzahl kleinerer Körnchen. Diese Körnchen sowohl als auch die grösseren Körnchen standen in inniger Beziehung zu den Fäden der Centralspindel." These granules then wander from both sides towards the equator of the spindle, "so dass sie . . . eine äquatoriale Körnchenplatte bilden. . . . Sobald die Einschnürung des Zellleibes bis zur Centralspindel vorgeschritten ist, werden die mehr peripher gelegenen Centralspindelfasern gerade im Aequator da, wo die Centralspindelkörperchen liegen, durchschnitten, und man sieht die Körperchen zugleich mit den verkürzten und undeutlich werdenden Fasern sich wiederum polwärts begeben." At each pole, then, these granules become

so densely grouped that often only one or two large granules appear to be present. "Mit der völligen Durchschnürung der Zellen wird schliesslich . . . der Zwischenkörper in zwei Theile getrennt, von denen jeder einer Tochterzelle angehört." Similar in the main points is also this process in the *Chick*, *Frog*, *Axolotl*, *Triton*, and *Salamandra:* "Wenn wir uns nun fragen, ob diese Vorgänge bei tierischen Zellen mit Recht mit den Vorgängen der Zellplattenbildung bei den pflänzlichen Zellen homologisiert wurden, so kann ich diese Frage nur zum Teil bejahen"; for two processes take place together, "nämlich eine äquatoriale Differenzierung der Centralspindelfasern zum Zweck ihrer Halbierung und eine eigentliche Zellplattenbildung, aus der die Zellscheidewand hervorgeht. Von diesen beiden Prozessen ist der eine, nämlich eine eigentliche Zellplattenbildung zum Zweck der Scheidewandbildung, bei tierischen Zellen gar nicht vertreten, wodurch der zweite desto deutlicher und unverhüllter zu Tage tritt." (Kostanecki mentions the following observations of previous authors on the occurrence of such a granular aequatorial plate in animals: Van Beneden, germs of *Dicyemida;* Balbiani, epithelial cells of an *Orthopterous* larva; Fol, eggs of echinoderms and *Cymbulia;* Flemming, eggs of echinoderms; Bütschli, egg of *Nephelis;* Mark, egg of *Limax;* van Gehuchten, egg of *Ascaris megalocephala;* Prenant, testicle cells of *Scolopendra* and *Lithobius;* Henking, similar cells of *Pyrrhocoris;* numerous observations of Carnoy; Van Beneden, ectoderm of vertebrate embryos; Strasburger, cartilage cells of vertebrates; Mayzel, corneal epithelium of *Fringilla;* Schleicher, cartilage cells of *Batrachia;* Carnoy, *Triton;* Bütschli, embryonal blood corpuscles of chick; Schottländer, inflamed epithelium of the cornea of the frog.)

Kraepelin ('92, cited by Braem, '97) noticed in the Bryozoan egg a division of the nucleolus.

Lönnberg ('92) studied the nucleoli of various ova and somatic cells. In the liver cells of *Mytilus* there are two "Nebennucleoli" and one "Hauptnucleolus." In the cells of the intestinal epithelium of *Tellina* a granule is sometimes found on the outer surface of the nucleus, which resembles a small nucleolus, and stains in the same manner. *Doris*, egg: "eine stärker

sich färbende Kugel (meist auch eine oder mehrere kleine Vacuolen) in einer grösseren hineingesenkt war und so den Nucleolus darstellte." *Mytilus:* "In den Einucleolen von *Mytilus* liegt oft eine (oder bisweilen zwei) grosse, blasse Kugeln in der Mitte oder ein wenig excentrisch, aber von der stärker sich tingirenden Substanz vollständig umschlossen; es ist schwer zu unterscheiden, ob es sich hier nur um Vacuolen handelt. . . . Bei *Aeolidia papillosa* [Ei] . . . zwei, ein wenig abgeplattete Kugeln die in einanden teilweise eingesenkt sind. Diese Kugeln sind aber hier beinahe gleich gross und die blasse ist in der gefärbten eingesenkt, bei *Unio* [nach Flemming] umgekehrt. . . . In den jungen Keimzellen fand ich nur einen einfachen Nucleolus, und dieser färbte sich stark." In the liver cells of *Doris proxima* there are two nucleoli: "Der eine von diesen ist ganz kugelrund und stark lichtbrechend, glänzend; dieser, der sich auch intensiv tingiert, muss als eigentlicher Nucleolus aufgefasst werden. Der andere ist blasser und grösser, seine Gestalt ist bald rundlich, bald länglich, bohnenförmig also mehr unregelmässig; diesen möchte ich als Nebennucleolus bezeichnen"; the two stain differently; "Die Lage der beiden Kernkörperchen ist auch wechselnd, indem sie bald ganz neben einander liegen oder sogar der Nucleolus im Nebennucleolus hineingesenkt, bald völlig getrennt sind. . . . Der Nebennucleolus, der immer scharf begrenzt ist, enthält oft eine kleine Vacuole. Ein Paar Mal traf ich in demselben Kern zwei Nebennucleoli." The latter are homogeneous, with an outer clearer layer, while the "Hauptnucleolus" is granular. Lönnberg found similar nucleoli also in the liver cells of *Polycera* and *Aeolidia.* Liver cells of the "*Krebs*" (*Astacus?*): "Meist sieht man . . . einen blassen Körper, der sich schwach wie der Nebennucleolus bei den Nudibranchiaten färbt, und daneben einen oder mehrere kleine Körperchen, die sich intensiv tingiren und sich wie Nucleolen verhalten; . . . bald liegt ein stark gefärbtes Kügelchen an einem Pole des Nebennucleolus, bald eins an jedem Pole desselben und in wieder anderen Fällen schmiegen sich drei Nucleolkörperchen dem Nebennucleolus an. Bisweilen treten Nebennucleolen in zwei- oder dreifacher Zahl auf." Lönnberg concludes

that the "Nebennucleoli" may play a part in the acquisition of nourishment or may hold reserve nourishment.

Marshall ('92) studied the sporulation of *Gregarina blattarum* v. Sieb. In the youngest individuals there is one large nucleolus. In larger ones there are one large and two or three smaller nucleoli, or four or five smaller ones of equal size; these now increase in size, accompanying the growth of the nucleus. He believes that the smaller nucleoli which are subsequently produced, arise in only one (as a rule) of the four or five original nucleoli: "Im Innern dieses Formationsnucleolus erscheinen dann klare, runde Ballen von verschiedener Grösse, welche keine bestimmte Grösse haben. Sie sind in wechselnder Zahl vorhanden und etwas heller als die übrige Masse des Nucleolus. Bei vielen Formationsnucleoli . . . waren alle Stadien der Entwickelung zu finden; kleine und grössere Ballen im Innern, und einige, die schon halb nach aussen getreten waren." After leaving the "Formationsnucleolus" they stain like the latter, and become either irregularly or spirally grouped together. "Die Vermehrung dauert bis zum Beginn der Cystenbildung fort. . . . Am Anfang der Encystierung enthält jeder Kern etwa 25–40 deutlich erkennbare Nucleoli, welche bald in dieser, bald in jener Art angeordnet sind. In beiden Fällen liegt der jetzt unregelmässig gestaltete Formationsnucleolus der von ihm ausgegangenen Gruppe gegenüber"; the latter is smaller than heretofore, "doch zeigt er noch Ballen im Innern." The smaller nucleoli increase in number, but now by repeated divisions of their own; the small granules resulting from these divisions are termed "Chromatinkörner": "Jedes Chromatinkorn bildet nun eine Hülle um sich, nachdem es sich vorher mit einer Schicht Plasma umgeben hat. Auf diese Weise vollzieht sich die Bildung der jungen Sporen. . . . Kurze Zeit, nachdem die Spore gebildet ist, nimmt dieses Chromatin-Korn die Gestalt einer 8 an und teilt sich in zwei Hälften, die beide an die entgegengesetzten Seiten der Spore treten." Later each of these divides into two, and each of the resulting four then divides into two, so that eight is the result; then one such "Chromatin-Korn" is allotted to each "Keim" (young Gregarine) and represents its nucleus.

Rückert ('92) studied the maturation of the eggs of *Scyllium*, *Pristiurus*, and *Torpedo*. In young germinal vesicles there are a few small nucleoli, most of them peripheral in position. In larger ova they have increased in number and size, and become grouped in a cluster at that part of the nucleus which is nearest the animal pole of the egg; this cluster may occupy one-fourth of the whole space of the nucleus. Later, but still antecedent to the formation of the pole spindles, the nucleoli decrease in size and commence to stain very faintly. Rückert considers the nucleolus of an egg cell as strictly comparable to that of any somatic cell. From the fact that the nucleoli are largest, and color most intensely, at the same time that the chromosomes do, and simultaneously with the latter become gradually invisible later, he concludes: "dass es die Stoffwechselvorgänge der Chromosomen sind, zu welchen die Nucleolen in direkter Beziehung stehen, sei es nun, dass sie notwendige Stoffe an die letzteren abgeben (vielleicht das Chromatin, wie schon Flemming vermutete), oder dass sie Stoffe von ihnen aufnehmen, oder endlich dass beides zugleich der Fall ist. . . . Später freilich, wenn die Chromosomen merklich an Substanz verlieren, wird man eher geneigt sein, die betreffenden Nucleolen als Träger von Zerfallsprodukten der Chromosomen anzusehen." He also observed that the number of the nucleoli varies in different germinal vesicles of the same age, that a number may coalesce to form a larger one, and that a few wander out into the cytoplasm, where they become paler and finally vanish.

Wirén ('92) found that the smallest germinal vesicles of *Chaetoderma* contain no nucleoli; in nuclei of about 15$\mu$ diameter a nucleolus appears for the first time, and consists of a dense mass of granules, which stain differently from the other nuclear granules. More than one nucleolus is never to be found.

*1893.*

Van Bambeke ('93) found one to five homogeneous nucleoli in the germinal vesicles of *Scorpaena scrofa*, and notes that in older eggs they do not stain as deeply with carmine as in younger ones.

Böhmig ('93), *Rhodope veranii:* the single nucleolus in older eggs contains one or several vacuoles.

Brauer ('93) investigated the spermatogenesis of *Ascaris megalocephala:* there is one homogeneous nucleolus in the spermatogonium, which becomes smaller in the spermatocyte and often evinces a large vacuole. The nucleolus is smaller than the centrosome (which is at this stage enclosed in the nucleus), and stains differently from the latter.

Brooks ('93), *Salpa:* the single large nucleolus of the ovarian ovum "is suspended near the center of the nucleus by a network of fine threads."

Fick ('93) studied the maturation of the egg of the *Axolotl.* In the germinal vesicle lies a group of nucleoli, which vary in size from 3$\mu$ to 16$\mu$; some contain a single vacuole, and some stain more deeply than others. The greater number of them disappear at the time of the longitudinal division of the chromosomes, though a few may remain in the yolk for a certain time. "Bei den Nucleolen des Keimbläschens liegt es sehr nahe mit Strasburger und Pfitzner daran zu denken, dass sie vielleicht eine Art Reservestoffbehälter darstellen"; further, he holds that the nucleoli "in einer allerdings noch nicht aufgeklärten Beziehung zu den Veränderungen des Chromatins stehen, da sie bei der Ausbildung der Chromosomen für die erste Spindel vollständig verschwinden."

Frenzel ('93a) studied the nucleoli of various *Gregarines.* In *Gregarina statirae* the single nucleolus, which he terms "Morulit," appears "eigenthümlich glänzend mit einem schwach gelblichen Schimmer und dabei an der Oberfläche rauh und warzig-runzelig. . . . In seinen Reaktionen verhält er sich an allen Orten ähnlich wie Nuklein." In *G. bergi* a single Morulit is present. In the embryo of *Pyxinia crystalligera* there is a single Morulit; in older individuals the nucleus "enthält mehrere helle, klare, glatträndige und lebhaft glänzende Nucleoli . . . die oft noch einen Flüssigkeitsraum im Innern bergen." In *Gregarina portuni, Callyntrochlamys,* and *Aggregata portunidarum* there are several nucleoli in the nucleus.

Frenzel ('93b) hepatopancreas cells of *Astacus:* in the fat and ferment cells a single nucleolus is present; in the amitosis

of the nuclei he concludes that the nucleolus divides ("nukleoläre Kernhalbierung"), since the nucleoli of the daughter-cells are of equal size.

Häcker ('93a) divides the maturation stages of the ovarian eggs of *Moina*, *Cyclops*, and *Sida*, into two periods, "von denen der erste gekennzeichnet ist durch die Anwesenheit eines einzigen 'Nucleolus' und durch die leichte Färbbarkeit des Fadenspirems (chromatische Stufe), der zweite durch die Anwesenheit mehrerer 'Nucleolen' und die Abneigung der chromatischen Substanz, die Mehrzahl der Färbungsmittel anzunehmen (achromatische Stufe)." In the first period ("Wachstumsphase") there is one excentric, deeply staining nucleolus ("Hauptnucleolus"), which possesses a "Hüllmembran"; in the second period, in addition to the "Hauptnucleolus" there are also one or two "Nebennucleoli" of greater size than the former, but staining less deeply, and somewhat irregular in form. Both kinds of nucleoli contain vacuoles. The "Nebennucleolus . . . stellt sich vielfach als hohles Gebilde von ellipsoidischer Gestalt dar, dessen einem Pole der Hauptnucleolus kappenförmig aufsitzt." Only the outer shell of this nucleolus stains deeply. Subsequently the "Hauptnucleolus" grows gradually smaller and finally disappears; and at the same time the "Nebennucleolus" increases in size and becomes irregularly lobular in shape, and finally breaks into pieces. The nucleolar relations in *Moina* are as in *Cyclops* (just described). In *Sida* only a "Hauptnucleolus" is present, and this contains a large central and several smaller peripheral vacuoles. Häcker distinguishes the following types of ova with regard to their nucleolar structure: (1) *Lamellibranchiate type*, with one "Hauptnucleolus" and one or two "Nebennucleoli," the latter larger and less chromatic than the former, but both frequently in close connection (*Naja*, *Anodonta*, *Cyclops brevicornis*); (2) *Echinoderm type*, with one large "Hauptnucleolus," which increases in size, and only towards the close of the "Keimbläschenstadium" do a few smaller nucleoli appear (*Toxopneustes*, *Sida crystallina*, primiparous *Cyclops strenuus* and *C. signatus*); (3) *Vertebrate type*, with several nucleoli varying in size, number, and form (*Rana* and

numerous other *Vertebrates, Sagitta, Moina, Cyclops brevicornis,* multiparous *C. strenuus*). "Aus der obigen Zusammenstellung . . . geht . . . hervor, dass das unter der Bezeichnung 'Nucleolus' oder 'Keimfleck' im Eikern auftretende Gebilde hauptsächlich in zweierlei Gestalt auftritt: entweder stellt dasselbe einen in der Einzahl vorhandenen, stetig seine Grösse verändernden, formbeständigen Körper dar, oder aber finden sich als 'Nebennucleolen' Bläschen oder Tröpfchen von wechselnder Zahl, Grösse und Gestalt vor." The "Hauptnucleolus" remains in the nucleus until just before the formation of the first pole spindle; after that it either diminishes rapidly in size, or it passes out of the nucleus into the cytoplasm, where it remains for a time as a "Metanucleolus"; the "Hauptnucleolus" is phylogenetically derived from a "Nebennucleolus," and has developed into "einen membranumhüllten, formbeständigen und stetig durch Diomose wachsenden Organ." "Es dürfte vielleicht zunächst die Thatsache heranzuziehen sein, dass ein Auftreten von 'Nebennucleolen' von wechselnder Zahl, Form und Grösse und von analoger chemischer Reaktion auch in den ruhenden Furchungskernen der betreffenden Thierformen festzustellen ist, und dass diese 'Nebennucleolen' hier nicht mit einem als Hauptnucleolus anzusprechenden Körper vergesellschaftet sind. Nebennucleolen treten folglich auch da im ruhenden Kerne auf, wo kein Zellenwachsthum stattfindet." Häcker considers that the "Nebennucleoli" are not drops of a nutritive fluid, but "Abspaltungsprodukte oder Sekretstoffe der chromatischen Substanz. Diese Auffassung findet vor allem in der Thatsache eine Stütze, dass die Nebennucleolen, z. B. bei *Moina* und *Cyclops strenuus* (mehrgebärend), im Lauf der Wachsthumsphase stetig an Grösse und Massigkeit zunehmen und dass sie das Maximum ihrer Entwickelung erst in dem Moment erreichen, wenn bereits die Vierergruppen zur Ausbildung gelangt sind, wenn also von einem Wachsthum der chromatischen Substanz kaum mehr die Rede sein kann."

Häcker ('93b): a preliminary contribution to the following paper.

Häcker ('93c) found in the germinal vesicle of *Echinus microtuberculatus*, in addition to the "Hauptnucleolus," a few small

globules which stain in the same manner as, and are probably homologous to, the "Nebennucleoli" of other animals; the "Hauptnucleolus" increases in size by the absorption of these latter. "Der Hauptnucleolus des Echiniden-Keimbläschens ist . . . ein pulsirendes Organulum, in welchem periodisch eine grosse Hauptvacuole sich durch Zusammenfluss kleinerer Vacuolen bildet, um dann wieder langsam abzunehmen. . . . Was die Dauer der Perioden anbelangt, so wurden solche von vier bis zu solchen von acht Stunden beobachtet"; the central vacuole at the time of its maximum size passes from the center to the periphery of the nucleolus: "Die Centralvacuole tritt also in Beziehung zur äussersten Wandschicht des Hauptnucleolus, anscheinend um ihren Inhalt mit . . . den Kernsaft in Kommunikation zu bringen." Accordingly, the "Hauptnucleolus" may be "als ein osmotisches System betrachtet werden, in welchem die feste Substanz (Rindensubstanz) nach zwei Seiten hin, einerseits mit dem Kernsaft, anderseits mit den Vacuolen, in diosmirender Verbindung steht. Sobald jedoch ein Körper nach zwei Seiten diosmirt, so ist eine Anhäufung in demselben nur durch das Eingehen einer neuen Verbindung möglich [Pfeffer ('91)]. Es folgt schon hieraus, dass die aus dem Kernsaft aufgenommene Flüssigkeit in der Nucleolarsubstanz nicht nur eine Verdichtung, sondern auch eine weitere chemische Umsetzung erfahren muss." The fluid vacuoles of the "Hauptnucleolus" represent an excretion which in *Echinus* is periodic, while in the *Copepoda* "im Laufe der Eireife wächst unter Mitwirkung der Rindenvacuole die Centralvacuole langsam heran, nimmt allmählich eine excentrische Lage an und entleert sodann kurz vor der Bildung der Richtungskörper ihren Inhalt nach aussen." He compares the vacuole of the "Hauptnucleolus" to the pulsating vacuole of the *Infusoria*: "so würden die Centralvacuole des Hauptnucleolus mit der eigentlichen pulsirenden Vacuole des Protozoenkörpers, die Rindenvacuolen des Hauptnucleolus mit den Bildungsvacuolen zu vergleichen sein." From a study of the pole-body mitoses he concludes: "dass der Hauptnucleolus während der Auflösung der Keimbläschenwandung zunächst noch in seiner ursprünglichen Grösse erhalten bleibt und sich

von dem Kernplasma langsam zu trennen beginnt." He could not exactly determine how long the nucleolus remains in the egg after that, but considers the fact important, "dass der Hauptnucleolus zur Zeit der Umbildung der Keimbläschensubstanz ohne bemerkbare Volumverminderung fort besteht"; it is at this time (after the disappearance of the nuclear membrane), to use his terminology, a "Metanucleolus."

Henneguy ('93) studied principally the genesis and occurrence of the yolk nuclei, "corps vitellin de Balbiani," in the ovarian egg of various vertebrates. This body is absent in the ova of the *Rabbit, Bitch, Mole, Rhinolophus, Cow, Antelope, Baboon;* and in *Lizards, Galeus, Raja,* and *Scyllium.* In the rat it consists of a peripheral clearer portion and a central denser core; it stains with eosin, safranin, and haematoxylin, but not with methyl green or gentian violet. In the bat it encloses a spherical corpuscle. It is also present in the chicken. Though absent in *Bufo* and *Triton,* it is found in *Rana temporaria,* where it is much the same as in birds, enclosing a more deeply staining portion. In the trout the corpuscle of Balbiani is as in the rat, but larger (twenty). *Sygnathus:* the very young egg contains a nucleus "renfermant un réseau chromatique bien développé" (his Fig. 20 would show three small nucleoli enclosed in this "réseau"); in older eggs the nuclear membrane is lined by a large number of nucleoli, and "le centre du noyau est occupé par une petite masse finement granuleuse et teintée en rose par la safranine, tandisque le reste du contenu demeure incolore. Le protoplasma ovulaire est également faiblement coloré et renferme un corpuscule arrondi, réfringent comme les taches germinatives et retenant la safranine avec la même intensité que ces dernières"; this corpuscle at the time of its first appearance is flattened against the outer surface of the nuclear membrane. Subsequently this intravitelline body becomes elliptical in form, with its long axis parallel to the surface of the egg: "il est de plus au contact immédiat par son bord externe avec une amas arrondi, constitué par une substance fondamentale d'apparence homogène, mais remplie de granulations très colorées. A un stade plus avancé tout le corps réfringent s'est transformé en un amas let qu'on l'observe dans

la plupart des ovules des Poissons"; in this manner it develops into a Balbianian corpuscle, and later breaks into small granules. In no eggs of any of the species studied are more than one of these corpuscles present; and it always arises during the maturation period of the ovum, before fecundation. "C'est très probablement une partie de la tache germinative, ou une tache germinative entière, qui sort la vésicule [germinative] pour pénétrer dans le vitellus. . . . C'est un organe ancestral qui, avec les éléments nucléolaires de la vésicule germinative, correspond au macronucleus des Infusoires, le micronucleus étant représenté par le réseau chromatique, prenant seul part aux phénomènes de fécondation."

Heuscher ('93) noticed in the ovum of *Proneomenia* either one nucleolus, or two of different size which were usually separated from each other.

Holl ('93) studied the maturation of the ovum of the mouse. "Die Fäden [Chromatin] zeigen eine innige Verbindung mit dem Kernkörperchen derart, als wäre dasselbe ein Centrum, von welchem die Fäden des Netzwerkes auslaufen." The nucleolus is not homogeneous, but contains granules ("Schroen'sche Körner") to the number of twenty; these gradually become stained during the growth of the nucleus, until the whole nucleus becomes evenly stained. "Im weiteren Verlaufe der Entwickelung treten die Schroen'schen Körner aus dem Kernkörperchen heraus und gelangen als chromatische Ballen in das Kernnetz, wo sie sich mit den Fäden desselben verbinden. . . . Endlich wird das Kernkörperchen von seinem Inhalte ganz frei; es bleibt nur die Kernkörperchenmembrane übrig, und im Kernraume liegen zerstreut eine grössere Anzahl der chromatischen Ballen. Dieselben sind anfangs klein und schwach gefärbt, wachsen auf $2\mu$ heran und färben sich immer besser. . . . Die chromatischen Ballen wandern aus dem Kerne aus, und das übrige [Fadennetzwerk] rückt als 'Kernrest' ganz an die Oberfläche der Eizelle. Die chromatischen Ballen liegen in 6 Gruppen von je 4 neben einander, und jeder Ballen wandelt sich in eine dicke, kurze Schleife um," *i.e.*, a chromosome of the "Richtungsspindel."

Jordan ('93) studied the development of the ovum of the

newt. He thinks "that certain deutogenic substances are formed in the [germinal] vesicle, perhaps through the agency of the nucleoli, and are then sent forth to share in the building up of the cell," *i.e.*, of the yolk particles. "The nucleoli in the young egg appear arranged along the chromatin threads, and possibly originate from the thread substance." Later they lose this connection, grow larger, and assume a peripheral position within the nucleus. There is apparently no division of the nucleoli; they "attain their maximum size shortly before their centripetal movement." Having arrived at the periphery of the nucleus, the nucleoli commence to stain less deeply, their contours become uneven, and they then wander back to the center of the nucleus, where they disintegrate. He does not agree with O. Schultze ('87) that the nucleolar particles build up the chromosomes.

Kaiser ('93) found in the egg of *Echinorhynchus bipennis* one large, spherical, peripherally situated nucleolus. It disappears before the pole spindle is produced.

Lustig and Galeotti ('93), mentioned by Lardowsky ('94), consider that the centrosome does not proceed from the nucleolus.

Mertens ('93), ovum of *Homo:* two or three nucleoli are present, consisting of a central clearer and a peripheral darker portion; it is probable that several smaller ones may fuse together to form a larger one; they are at first in intimate connection with the chromatin filaments, but later lose this connection and gradually cease to stain with safranin. The Balbianian corpuscle is an extruded nucleolus: "c'est alors aussi que nous nous étondrons quelque peu sur l'expulsion des parties chromatiques du noyaux, expulsion qui paraît affecter les mêmes charactères chez les oiseaux et les mammifères"; eliminated nucleoli ("grains chromatiques") as well as attraction spheres have been described as Balbianian corpuscles. Ovum of *Pica:* in young ovules there is one nucleolus which arises as follows: at one point in the nucleus the reticulum concentrates itself, and here a certain number of the filaments fuse together, thus producing the nucleolus. The chromatin is at first irregularly arranged in the nucleolus, but "finit par être également dense dans toutes les parties de la tache germinative," and subse-

quently accumulates on its surface. "Le nucléole devenu indépendant [from the chromatin reticulum] est expulsé : les chromosomes s'écartent pour lui livrer passage. Il n'est pas rare d'en rencontrer qui, arrivés à la périphérie, sont coiffés par un filament nucléinien. . . . Le filament se rompt bientôt et le nucléole est libre." The presence of a vacuole in the nucleolus is explained by the assumption that the chromatin wanders to the periphery of the nucleolus, thereby leaving a clear space at the center of the latter. (Safranin the only stain employed.)

Minchin ('93) states that the single nucleolus of *Gregarina irregularis* "consists of a darkly stained ground substance containing an immense number of clear vacuoles of all sizes. One of the vacuoles is much larger than the others, and being excentrically placed, constitutes the clear spot seen in the thick sections." The nucleolus of *G. holothuriae* has a similar structure.

Pizon ('93), ova of *Botryllida:* a single large nucleolus containing several vacuoles.

Repiachoff ('93) figures a large vacuole in the single nucleolus of the ovarial cells of a pelagic, acoelic *Rhabdocoele* (species undetermined).

From Rhumbler's contribution ('93) to the morphology of the nucleoli, or "Binnenkörper," the following extracts are important: "Mir scheint es . . . noch keineswegs sicher, ob die Nucleolen der Gewebszellen und die Nucleolen der Keimzellen bezw. vieler Protozoen (vielleicht ausgenommen die Ciliaten und Suctorien) analoge Gebilde sind; obgleich auch das Gegentheil wegen des ähnlichen Verhaltens der beiderlei Nucleolenarten während der Mitose sehr zweifelhaft bleiben muss." In *Saccamina sphaerica* there are from 1 to 300 nucleoli: "ähnlich wechselnd wie ihre Zahl ist ihre Grösse, ihr Lichtbrechungsvermögen und ihre Gestalt." The largest of them "zeigen meist eine, durch stärkeres Lichtbrechungsvermögen ausgezeichnete Innenmasse, in welche kleinere, noch stärker brechende und oft von der Kugelgestalt abweichende unregelmässige Körperchen eingelagert sind, und eine dunklere, weniger lichtbrechende Aussenmasse, die in gleichmässiger Dicke wie

eine feste Membran um die Innenmasse herum gelagert ist"; this latter portion also stains more intensely with eosin. Rhumbler concludes that the "Binnenkörper . . . durch Zusammenfliessen anfänglich leicht flüssiger, dann zähflüssiger und schliesslich erstarrender Massen entstanden sind. Ich nehme an, dass die Binnenkörpersubstanz an allen oder auch nur an bestimmten Stellen (das Letztere da, wo eine fixirte Nucleolenzahl Regel ist) des Kernplasmas zuerst in Gestalt kleinster, erstarrender Tröpfchen abgeschieden wird, die auf verschiedenen Stadien ihrer Erstarrung an einander treffen," this deduction being based in part on an observation of A. Schneider ('75). He explains why the nucleoli are not evenly distributed in the nucleus, on the ground "dass die einzelnen Tröpfchen jedenfalls nicht an allen Stellen des Kernraumes zu genau derselben Zeit entstehen." The nucleoli probably represent "Reservestoffe," which are consumed in the later growth of the nucleus, and since in *Saccamina* they decrease in size as the amount of the chromatin increases, it is probable "dass die Nucleolensubstanz [die sehr verschieden sein kann] in irgend welcher Beziehung zum Chromatin steht." Further, he holds that the nucleolar substance is produced in the nucleus, "und dann erst erzeugt wird, wenn sie in kleinen Tröpfchen auftritt." But it is not yet possible to decide whether the nucleoli of the *Metazoa* also arise in this manner, and hence the use of the general term "Binnenkörper" instead of the more specific one "Nucleolus." That amoeboid movements of nucleoli have been noticed is not contradictory to his theory, since changes of form would be caused by the processes of fusion, or these motions might denote "Auflösungsvorgänge": "Die Auflösung der Binnenkörper muss nach unserer Annahme von zwei, im Kernsaft enthaltenen, sich gegen die Binnenkörper konträr verhaltenden Substanzen, auf eine Ueberschreitung des angestrebten Mischungsoptimums von Seiten der lösenden Substanz zurückgeführt werden. . . . Der Verschmelzungsvorgang ist schon von mehreren Forschern erschlossen oder vermuthet worden — neu dürfte nur die Annahme einer allmählichen oder auch rascheren Erstarrung der ursprünglich flüssigen Binnenkörpersubstanz sein." Rhumbler concludes that the

"Binnenkörper" are not organs, since they show no fixed organic structure, but represent accumulations of various substances. There is more nucleolar substance, "Reservestoff," accumulated in the nucleus before mitosis than is necessary for it, so that after a mitosis some always remains to serve for the production of daughter-nucleoli (this being an explanation for the reappearance of nucleoli after mitosis).

Stauffacher ('93), maturation of the egg of *Cyclas:* the "Urei" contains a single large nucleolus; later one or two "Nebennucleoli" also appear in the nucleus. When the ovum has so increased in size that it adheres to the wall of the ovary only by a narrow thread of cytoplasm, two nucleoli are present, which are of unequal size but are in close contact with each other; in one case the nucleolus was trilobular. After borax-carmine staining, the smaller one appeared more refractive and deeply stained than the larger. Subsequently the two became separated, and both vanished before the formation of the first pole spindle.

Strasburger's paper ('93) presents a general discussion of certain problems of mitosis in animals and plants; his remarks on the aequatorial plate are apropos here. He believes that the "Körnchen" found by Kostanecki ('92) in the equator of the central spindle are similar to, and comparable with, structures found by himself in the mitoses of plants, and are masses of nucleolar substance (these bodies being termed "Centralspindelkörperchen" by Kostanecki, "Zwischenkörper" by Flemming, and "Zwischenkügelchen" by O. Hertwig, '92). "Vergegenwärtige ich mir nun das, was ich seinerzeit bei der Bildung pflanzlicher Zellplatten beobachtet habe [*Histol. Beitr.*, vol. i, p. 161], nämlich das Fortschreiten jener tingirbaren Substanz, die ihrem Auftreten und ihren Tinctionen nach nur als Nucleolarsubstanz gelten konnte, zwischen den Verbindungsfäden bis zum Aequator, so muss in mir die Vorstellung erwachen, dass es sich in der von v. Kostanecki geschilderten Erscheinung um einen entsprechenden Vorgang handle. . . . Mit den durchschnittenen [achromatischen] Verbindungsfäden . . . wanderte dann auch die Substanz der halbirten Zwischenkörper nach den Zellkernen zurück, ähnlich,

wie wir das für die unverbrauchte Nucleolarsubstanz bei Pflanzen angeben konnten. . . . Bei Pflanzen treten die Elemente der Zellplatten als Anschwellungen der Verbindungsfäden im Aequator der Zelle auf. Diese Anschwellungen bilden sich dort erst, wenn jene tingirbare Substanz . . . den Aequator erreicht. Diese Substanz wird in gelöster Form zwischen den Verbindungsfäden dorthin befördert. Aus den verschmolzenen Elementen der Zellplatte geht die Scheidewand hervor. . . . Man könnte denken, dass in tierischen Zellen ein mittlerer Teil der 'Zwischenkörper' in eine lösliche Substanz sich verwandle und so die Halbierung der Zwischenkörper und damit auch der Verbindungsfäden bewerkstelligte."

Ver Eecke ('93), pancreas cells of *Rana* and *Canis:* he distinguishes one "nucléole nucléinien" and several "nucléoles éosinophiles," or plasmosomes, the latter being the larger. When the cell enters on its functional activity "le plasmosome unique devient plus volumineux; il n'est pas rare d'en trouver plusieurs dans un seul noyau; ils se rapprochent de la membrane nucléaire, la soulèvent, la perforent et se placent en définitive a côte du noyau pour former un noyau accessoire. D'ordinaire le plasmosome dans sa migration est accompagné de petits karyosomes qui lui forment parfois une véritable couronne"; the mother-nucleus subsequently degenerates. Against the opinion of Platner (that the supposed migration of the nucleoli is artificially produced) "il suffit de faire remarquer que la migration ne s'observe pas ou très rarement à l'état de repos pour ne se manifester dans tout son éclat qu'au début de l'activité sécrétoire." In the cytoplasm the nucleolus and its attendant karyosomes gradually change into a nucleus.

Wasielevsky ('93) found the "Urgeschlechtszelle" of *Ascaras* with one or two nucleoli. While in the resting state of the nucleus only one nucleolus is present, two are regularly seen in the spirem stage, and these he believes have originated by division of the primitive one. He noticed no difference in size or stain between these nucleoli and the centrosomes, and hence concludes that the latter are identical with, or have some genetic relation to, the former.

*1894.*

Blochmann ('94) gives a preliminary account of the results of the observations of Keuten ('95).

Born ('94) investigated the maturation of the ovum of *Triton*. In the "Urei" are one or several large, spherical nucleoli. In the second stadium of the maturation (production and degeneration of a "Chromatinfadenknäuel") there are at first ten nucleoli, then they become more numerous, increase in size, and lie close to the nuclear membrane. In the third stadium (eggs of from 200$\mu$ to 350$\mu$ in diameter) the nucleoli increase still more in size. In the fourth stadium (eggs measuring from 350$\mu$ to 800$\mu$, first appearance of yolk in the cytoplasm) most of the nucleoli lie in the peripheral "Karyohyaloplasma," only a few pale ones being in the center of the nucleus (this part of the nucleus he terms "Centralkörper"). At the commencement of this stage the nucleoli increase, at its conclusion decrease, in number, and "während der ganzen Periode steigt die Zahl der verkleinerten und abgeblassten Nucleolen im Centralkörper," only a few of these pale ones being situated at the periphery of the nucleus. Thus while at the beginning of this period the nucleoli attain their maximum size, at its end most of them wander towards the center of the germinal vesicle, become smaller, and lose their staining power. Fifth stadium (the nucleus passes to the periphery of the egg): the nucleoli decrease still further in size, and continue to wander to the center of the nucleus; some of the larger ones contain vacuoles, and for the first time appear granular; the smaller, lightly staining nucleoli are division products of the larger ones. At the commencement of the sixth stadium (formation of the first pole spindle) all the nucleoli lie in irregular rows around the "Centralkörper," stain quite intensely, and are regularly vacuolated; the few in the midst of the "Centralkörper" are smaller and stain more faintly; when the nucleus has decreased still further in size, all the nucleoli vanish at once. Born concludes as follows: "Eine sichere Herleitung der peripheren Nucleolen von den Nucleolen des Ureies, bin ich freilich nicht im Stande zu geben. . . . Die Nucleolen stehen in Beziehung

zum individuellen Zellleben, nicht zur Fortpflanzung; denn beim Beginn der Mitose verschwinden sie, um nach Beendigung derselben — im Ruhestadium des Kerns — wieder aufzutreten." He notes that their peripheral position is "Eine Lage, die für eine Wirkung auf den Zellleib die denkbar günstige ist."

Brauer ('94), *Actinosphaerium:* in the cyst of the second order ("Ruhecyste") there is a nuclear reticulum consisting of chromatin granules imbedded in a linin network, and usually numerous nucleoli of irregular form, arranged either in rows or circles. Probably the nucleoli take no part in the formation of the chromosomes, and are equivalent to those of metazoan cells; they disappear in the prophase of mitosis.

Bunting ('94) found in the eggs of *Hydractinia* and *Podocoryne* a single large nucleolus, containing one central vacuole of large size.

Flemming's ('94) "Referat" includes some of the more recent papers on nucleoli.

Foot ('94), egg of *Allolobophora:* during the first maturation division the nucleoli are distributed in the cytoplasm. Each pronucleus contains from one to seven nucleoli: "the nucleoli persist during the cleavage spindle, but how much later I am unable at present to state."

Hodge ('94), nerve cells of *Rana* stimulated by the electric current: amoeboid movements of the nucleolus were noticed; "it was possible to make out granules in the nucleolus which moved slowly about and in several instances were seen to be extruded into the nucleus"; and in cells which had not been stimulated, but simply fixed in osmic acid and stained with safranin, "the granules were stained brighter red than the body of the nucleolus, and several were found partially extruded."

Lavdowsky ('94) studied nuclei from the epidermis of the fins of *Amphibian* larvae, as well as various tissues of plants. The nucleolus consists of: (1) an outer, thick "Pyrenin-Chromatinschale"; (2) an enclosed vacuole; and in the latter (3) the "Nucleololus" ("das noch in Entwickelung begriffene Centrosoma"). The animal nucleolus varies from a spherical to an angular or star shape. In the resting nucleus the chro-

matin and pyrenin shells are the largest, since "die Bestandtheile noch nicht für die Karyokinese verbraucht sind." The centrosomes "sind wahrscheinlich Teile von Kernkörperchen und wandern zur Zeit der Karyokinese von den Kernelementen aus" (these centrosomes are spherical or oval, homogeneous and compact, and stain very slightly). He concludes "dass die Kernkörper nicht zu jeder Zeit des Zellenlebens persistieren, dass ihr Verschwinden während der Karyokinese keinem Zweifel unterliegt und dass dies in innigem Zusammenhang mit dem Erscheinen des Centrosoma steht." The nucleoli divide amitotically (not seen in life, however) into very small pieces, which "scheinen in das Gerüstnetz eingeschaltet und verwandeln sich in den Vorbereitungsstadien der Karyokinese in Chromatinfäden"; other "Kernkörper" pass out of the nucleus, at the points where its membrane is broken. The nucleoli are not sufficient for supplying the whole mass of chromatin necessary for the mitosis; "es muss also eine andere Quelle der Chromatinentwicklung da sein und hauptsächlich im Eidotter und in den pflanzlichen Samen muss man die Quelle aufsuchen. . . . Durch nichts unterscheiden sich die Chromosomen von den zerteilten Dotterkörnchen und den geteilten Nucleolen. Alle diese Gebilde . . . können somit als 'Kariosomen' betrachtet werden."

Metzner ('94), cells in the testicle of *Salamandra:* he concludes "dass die Nucleolen in keinem Stadium der Mitose fehlen, obwohl sie von sehr verschiedener Grösse sind." In resting nuclei the smaller nucleoli stain entirely with gentian violet (after Flemming's triple stain), the larger ones with safranin except for a blue-stained peripheral zone. Smaller nucleoli are budded off from the surface of the larger ones, and the "Leitkörper" (granules which serve to attach the chromosomes to the spindle fibers) resemble such buds in stain and size; "es ist mir vorerst nicht möglich zu entscheiden, ob diese Leitkörper von dem Nucleolus stammen, doch ist es wahrscheinlich, dass gerade an ihm sich die ersten, den Kern- und Zelltheilungsprocess einleitenden Vorgänge abspielen. Denn an den Zellen mit ziemlich gleichmässiger Vertheilung der Chromatingranula und geringer Anzahl der Nucleolen kann

man immer schon den Vorgang der Ausstossung kleinster Kügelchen beobachten." In mitosis the nucleoli wander into the cytoplasm, where the larger of them disappear, while the smaller persist; "dass aber diese Nucleolen in den Tochterkern einwandern, ist nicht sehr wahrscheinlich, denn es liegen in den Tochterzellkernen nur die gelösten Leitkörper. . . . Vielleicht persistiren nur einige von ihnen in der jungen Zelle und zwar als Nucleolen. . . . Eine 'Vermischung von Nucleinsubstanz' kann ich . . . an meinen Präparaten . . . für Chromatingranulastrange nicht annehmen, denn die augenscheinlich von den Nucleolen stammenden Leitkörperchen adhäriren nur den Segmenten und erfüllen ihre . . . Function als Anheftungspunkte der Spindelfibrillen; sie lösen sich intakt in den Tochterknäueln wieder ab. Dass sie noch andere Functionen ausüben (als Nucleolen) ist wahrscheinlich, doch nicht ganz sicher. . . . An den Nucleolen treten die ersten Erscheinungen der Zelltheilung auf. Sie lassen aus sich eine Menge kleiner Kügelchen hervorgehen, die z. Th. aus dem Kerne in das Protoplasma wandern, z. Th. aber als Leitkörperchen über den Kern sich vertheilen und so wohl den Anstoss geben zur Strangbildung der Chromatingranula. Dem Nucleolus fiele also für die Fortpflanzung der Zelle eine wichtige Function zu."

Murbach ('94) considers it probable that the "Kapselkeim" of the nettle capsules of hydroids is derived from one of the two nucleoli of the parent cell, in accordance with his view that the capsule is of nuclear origin.

Purcell ('94) describes the nucleolus of the retinula cells of *Acantholophus* as structurally "wabig."

Reinke ('94) found in the cells of the spleen of the mouse one oval or elongate nucleolus; during the prophase of mitosis this divides into three or four smaller ones, while at the end of mitosis each daughter-nucleus has a single nucleolus.

Rückert ('94) studied the maturation of the ovum in three species of *Copepoda*. *Cyclops strenuus* (his species he assumes is not identical with the "C. strenuus" of Häcker): in the "Wachsthumszone" of the ovary there is one large, sometimes also two smaller nucleoli, which stain with haematoxylin as does the chromatin, and together represent the "Hauptnucleolus"

of Häcker. The single "Nebennucleolus" appears a little later, and is regular in its occurrence, both in females with egg sacks ("mehrgebärend," after Häcker) and in those without egg sacks ("erstgebärend," according to Häcker); Häcker found the "Nebennucleolus" only in the ova of the former category of females. It is paler and much larger than the several "Hauptnucleoli," and has a more central position within the germinal vesicle, while the latter are usually peripheral. When the "Hauptnucleoli" have disappeared the "Nebennucleolus" increases in size and thereby at first assumes a mulberry shape, or is produced into long processes (though at the start it was spherical). "Während er anfänglich ein kompaktes Gefüge besitzt, lockert er sich später auf. Schon frühzeitig sieht man in seinem Innern einen lichteren Raum, und später entrollt er sich zu knäuelartig gewundenen Zügen, die ein sehr wechselndes Ansehen bieten, sehr häufig bilden sie eine einzige, ziemlich einfach verschlungene Figur, ein Achtertour, ein S u. a., neben der jedoch noch ein oder ein paar kleinere kugelige Stücke im Keimbläschen liegen können. . . . Er ist . . . nicht einheitlich gebaut und homogen, wie ihn Häcker abbildet, sondern zusammengesetzt aus rundlichen Anschwellungen, die in einer Reihe hinter einander liegen, stellenweise getrennt durch schwach gefärbte, schmälere Zwischenstücke. Man könnte daher das Ganze als eine Kette von Kugeln bezeichnen. . . . In etwas späteren Stadien verlieren diese Bildungen an Färbbarkeit, erscheinen aber zunächst immer in sehr wechselnder Form. Man trifft entweder einen mehr kompakten Substanzhaufen oder meistens eine Anzahl durch das Keimbläschen zerstreuter Stücke. . . . Häufig sieht man ein vielfach verschlungenes, sehr unregelmässig angeordnetes Fadensystem. . . . Es ist schwierig zu entscheiden, ob die beschriebenen, sehr wechselvollen Bilder der Ausdruck nur für verschiedene Functionszustände des Nucleolus sind, oder für einzelne, zum schliesslichen Zerfall führende Entwicklungsstufen"; they disappear before the true maturation processes commence. In *Heterocope robusta* and *Diaptomus gracilis* there is a single large, vacuolated nucleolus; it disappears when the chromatin has arranged itself into "Vierergruppen."

Schaudinn ('94) finds in *Amoeba crystalligera* a large nucleolus, with "wabiger Struktur"; in the mitosis it divides into two equal parts.

Watasé ('94), in the course of his theoretical deductions as to the structure of the cell, concludes in regard to the nucleolus: "The nucleolus is not a permanent body in the nucleus. It may exist at one stage of the cell, and may disappear at the next. The micro-chemical reaction of the nucleolus is entirely different from that of the chromosome. It appears probable that three or more different bodies are included under the name of nucleolus. Indeed, one sees no reason why the inside of the nuclear membrane may not be used as a depository for some solid products of cell metabolism. . . . And thus some of the bodies included under the generic name of *nucleolus* may belong to the group of metaplasm."

H. V. Wilson ('94), *Tedanione foetida:* the youngest germinal vesicle contains a single, centrally placed nucleolus. Later there are two nucleoli, "which are invariably placed on opposite sides of the nucleus and adhere to the inner surface of the nuclear membrane. In eggs which have reached the adult size it is the rule to find either one nucleolus peripherally placed, . . . or the nucleus contains no nucleolus at all. It sometimes happens that an egg of full size is found with two nucleoli, but this is rare. From this evidence it would seem that the two nucleoli present in the developing egg are lost, one after the other, at the time when the egg reaches its full size. As to how the first of the two is lost, I have no evidence, but the second nucleolus may often be seen lying just outside of the nucleus in the yolk, . . . showing that it has been extruded from the nucleus." What Fiedler ('88) described as polar bodies in *Spongilla* are probably extruded nucleoli. In the egg of *Hircinia acuta* the nucleolar changes are as in Tedanione.

*1895.*

Balbiani ('95), reviewed by v. Erlanger in *Zool. Centralbl.*, 1895, macronucleus of *Spirochona:* the nucleolus of the authors arises in a vacuole of the chromatin, and is formed by the separation of microsomes which fuse together to form one

or two nucleoli. The nucleolus then wanders through the chromatin to take position in the center of the achromatic substance ; it combines the qualities of a true nucleolus with those of a centrosome. There is thus no fundamental difference between a nucleolus and a centrosome ; when it remains in the nucleus it has the value of the former, when in the cytoplasm it has the significance of a centrosome.

Böhmig ('95) noticed in the ovarial eggs of *Haplodiscus* that the nucleolus is at first small and homogeneous, while later it becomes larger, and one or more vacuoles appear in it.

Bremer ('95a), blood cells of *Testudo* and *Chelydra :* there is normally one paranuclear corpuscle to a cell ; "seiner Natur nach ist das Paranuclearkörperchen ein vom Innern des Kernes in das Diskoplasma [Cytoplasma] ausgewanderter Nucleolus oder vielleicht ein Nucleolusfragment, umgeben von einer dem Kerne entnommenen Hüllsubstanz. . . . Seine Grösse, die Schwierigkeit der Färbung und seine Lage sprechen für den nucleolären Charakter." In a second paper ('95b) he identifies this corpuscle with a centrosome, and states : "Hertwigs Vermuthung, dass ein Zusammenhang des Centrosoms mit dem Nucleolus existire, wird durch meine Beobachtungen wahrscheinlicher gemacht."

In Bürger's monograph ('95) of the *Nemerteans* the following statement in regard to the structure of the germinal vesicle is of interest : "Im Keimbläschen findet man ausser den intensiv färbbaren Körperchen, den Nucleolen, von denen meist zwei, ein grösseres und ein kleineres, vorhanden sind, ein Netzwerk feiner Fäden, in welche sehr feine Kügelchen aufgehängt sind."

Coe ('95), ova of *Cerebratulus lacteus :* "as the ovum increases in size its nucleus develops into the germinal vesicle which has many germinal spots, of which one or two are much larger than the others." In the mature ovum the nucleus "often contains a highly refractive germinal spot one-third as large as the vesicle itself."

Cunningham ('95), ovarial eggs of fishes : in the youngest ova there is a single large nucleolus, in older ova a number of peripheral ones ; the latter are produced in part by a division of the primitive nucleolus, in part by an increase in size of

"minute nucleolar granules" which were present in early stages. In contradiction to the view of Scharff ('88) he finds that no nucleoli wander out of the nucleus to form yolk globules.

Delage ('95) opposes the view that the nucleoli and the centrosomes are genetically related (as against the theory of Julin ('93 b) and Wasielevsky).

Galeotti ('95), embryonal cells of *Triton* and *Spelerpes* (fixation in Hermann's fluid with chloride of palladium substituted for chloride of platinum; stained for five minutes in sat. sol. acid fuchsine in aniline water at 60° C., then stained in 1½% sol. methylen green in equal parts of water and alcohol for three or four minutes): "Auf diese Weise erhält man roth gefärbt die Körnchen des Cytoplasma und alle Elemente des Kerns mit Ausnahme des Nucleolus . . .; gelblichgrün erscheint der protoplasmatische Grund der Zelle und lebhaft grün die basophilen Granulationen." In the pancreas cells of *Spelerpes* the green-stained nucleolus passes out of the nucleus and persists as "Nebenkern," which in the cytoplasm seems to increase by continued division; and from this he concludes "dass der Nucleolus ein endonucleares Arbeitsprodukt des Kernes ist, bestimmt aus der Kernmembran auszutreten und im Cytoplasma so umgeändert zu werden, dass er in Secretionsprodukte umgewandelt wird."

Häcker ('95) first describes the nucleolar relations in the eggs of *Canthocamptus*, and then gives expression to general views, based on his numerous previous observations, in regard to the nature of nucleoli. *Canthocamptus staphylinus:* in the smallest eggs there is one large nucleolus, which increases in size, but not to same relative extent as does the nucleus itself; subsequently vacuoles arise in it, one of which becomes a "Hauptvacuole"; smaller "Kernkörper" appear first when the chromatin elements commence to thicken; "wenn endlich die Kernsubstanz auf das Minimum ihres Volumens zusammengedrängt ist, so fehlt in der Regel jede Spur von nucleolärer Substanz." Then follows his general conclusions in regard to the physiology and structure of the nucleoli: "Die Nucleolen sind nach meiner Ansicht im allgemeinen als nicht strukturirte Gebilde aufzufassen. . . . Sie stellen als solche . . . ein Abspaltungsprodukt,

welches während der vegetativen Thätigkeit der Zelle und des Kerns in oder an den chromatischen Elementen zur Abscheidung gelangt und zu Beginn der Mitose aus dem Kernraum entfernt wird. Wie bei allen organischen Wachsthums- und Umbildungsprocessen, so würden . . . Sekret-Substanzen zur Abspaltung kommen, welche in Form eines Hauptnucleolus oder mehrerer Nebennucleolen auftreten. . . . Die Gründe, welche theils für die Auffassung der Nucleolen als nicht organisirter Stoffwechselprodukte sprechen, theils speciell darauf hinweisen, dass es im Kern entstandene und dem Kern verlassende secretartige Stoffe sind," are the following: (1) "Die bedeutende Entfaltung der nucleolären Substanz in den Kernen solcher Zellen, für welche eine intensive vegetative Thätigkeit angenommen werden muss (Keim-Mutterzellen, Drüsenzellen, Ganglienzellen, Wimperzellen), würde zum mindesten dafür sprechen, dass die Nucleolarsubstanz ein Stoffwechselprodukt darstellt, dessen Erzeugung in einem gewissen Abhängigkeitsverhältniss zur Intensität der vegetativen Leistungen von Kern und Zelle steht." He cites numerous cases to show that all germ cells with little yolk and with usually adequal cleavage have a large "Hauptnucleolus" (sponges, *Hydromedusae*, *Siphonophora*, *Acalephae*, *Ctenophora*, *Echinodermata*, *Copepoda*, *Tomopteris*); while all large ova with a considerable amount of yolk and with discoidal or superficial cleavage have numerous "Nebennucleoli" (most *Insecta*, many *Crustacea*, lower *Vertebrata*). He explains the time of the appearance of the "Nebennucleoli" in the egg of *Canthocamptus* in this way: "Zur Erklärung dieser Erscheinung ist anzunehmen, dass irgend welche die ganze Eizelle betreffenden Veränderungen physiologischer Natur, die um diese Zeit eintreten, die weitere Apposition der neu sich bildenden Nucleolarsubstanz an den Hauptnucleolus verhindern und das Auftreten mehrerer Verdichtungscentren hervorrufen, welche häufig nicht mehr das Färbungsvermögen des ursprünglichen Hauptnucleolus erlangen . . . vom rein morphologischen Standpunkt aus darf man aber wohl mit diesen in den Endstadien der Eibildung auftretenden Bildern jeden intermediären Keimbläschentypus vergleichen, welcher sich im Lamellibranchiaten-Ei vorfindet."

He notes that "die Bildung nucleolärer Substanz auch unabhängig vom Zellwachsthum in erheblichem Masse stattfinden kann. Bekanntlich treten nämlich auch in den zur Copulation sich anschickenden Geschlechtskernen Nucleolen auf, welche nicht selten beträchtliche Dimensionen annehmen, und dasselbe gilt für die Kerne der früheren Furchungsstadien. Hier ist von einem Zellwachsthum nicht die Rede." Accordingly, he concludes: (1) "dass die Menge der nucleolären Substanz in einem direkten Verhältniss steht zur Intensität der Wechselbeziehungen zwischen Kern und Zelle"; (2) "hier möchte ich nur wiederholen, dass ich aus den verschiedenen Bildern eine Entstehung der Substanz der Nucleolen an oder in den Chromatinschleifen und die Möglichkeit einer Verschmelzung derselben ableiten und mich so entschieden gegen die Auffassung aussprechen möchte, dass die Kernkörper aus dem Zellplasma in den Kern hereingelangen und hier in die Bildung des Chromatins eingehen, sowie im allgemeinen dagegen, dass die kleinen durch Theilung der grösseren entstehen"; (3) he brings a few observations to show "dass der Kern die nucleoläre Substanz an das Zellplasma abgiebt, dass es sich also hier wohl kaum um Stoffe handelt, welche als Nährmaterial dem Chromatin zugeführt werden, sondern um solche, die während der Veränderungen des letzteren zur Abspaltung und dann zur Ausscheidung aus dem Kerne kommen. . . . Ich denke . . . , dass sie [die vorhergehenden Erörterungen] in ihrer Gesamtheit sehr wohl eine Stütze für die Kernsekret-Theorie bilden können." Finally, Häcker gives his own explanation of the maturation stages of *Triton*, based on the description of Born ('94), and, comparing the changes here with those observed by himself in the maturation of *Canthocamptus*, generalizes the two as follows: 1. Stadium (growth of the germinal vesicle), "Abscheidung einer dunkel tingirbaren Nucleolarsubstanz"; 2. Stadium, "Verdichtung der chromatischen Substanz und Concentrierung in die Kernmitte. Beginn der Auflösungsvorgänge. Der neu sich bildende Nachschub an nucleolärer Substanz erlangt . . . nicht mehr das ursprüngliche Färbungsvermögen"; 3. Stadium, "Grössenreduktion des Keimbläschens: Die chromatische Figur liegt unmittelbar im Zellplasma."

Held ('95) finds that in the ganglion cells of vertebrates, when stained with erythrosin followed by methylen blue, the nucleolus stains blue and the "Nebennucleoli" violet.

Herrick ('95) found that the nucleolus of *Homarus* contains one large and several smaller vacuoles; the gravitation of the nucleolus in the caryolymph, *i.e.*, its movement to the lower side of the nucleus, may be post-mortem phenomena (at least I learned as much from Dr. Herrick during a brief conversation).

Keuten ('95) investigated the nuclear division of *Euglena viridis*. In the nucleus there is an elongate body, the "Nucleolo-Centrosoma," which stains more intensely than any other portion of the nucleus. At the commencement of mitosis it elongates, "und während die Segmente [Chromosomen] bisher eine annähernd senkrechte Richtung zur Oberfläche des Nucleolo-Centrosomas eingenommen hatten, bilden sie jetzt einen spitzen Winkel mit demselben," and gradually come to lie parallel to it. At this time the middle piece of the "Nucleolo-Centrosoma" stains more lightly than its ends, so that these latter parts are sharply demarcated from it (with the stain of Heidenhain, namely, Bordeaux R. followed by haematoxylin). "In der folgenden Phase rücken die parallel zum Nucleolo-Centrosoma gelagerten Chromosomen von beiden Polenden her nach dem Äquator zu, so dass die Enden des Nucleolo-Centrosomes nunmehr frei in die Kernhöhle hineinragen, während die Chromosomen als breite äquatoriale Zone das Mittelstück des Nucleolo-Centrosomes umgeben." Next, the nucleus assumes the form of a rotation ellipse, in the short axis of which the "Nucleolo-Centrosoma" lies. After the longitudinal splitting of the chromosomes, from three to five vacuoles appear in each end of the "Nucleolo-Centrosoma"; then the latter structure elongates and breaks into two parts, while at the same time the long axis of the nucleus gradually changes so as to coincide with the long axis of the "Nucleolo-Centrosoma," and part of the chromosomes become grouped around the one end, the remainder around the other end, of the "Nucleolo-Centrosoma." Keuten believes his "Nucleolo-Centrosoma" to be comparable to the nucleolus of *Amoeba crystalligera* (Schaudinn), to the "Centralspindel" in *Diatomea* (Lauterborn), and to the centro-

some plus central spindle of *Ascaris megalocephala;* it is probably an important mechanical factor in the mitosis.

Korschelt ('95) finds that in the amitosis of the intestinal cells of *Ophryotrocha puerilis* the "Kernkörper" divides into two. Ovarial and cleavage stages of the same annelid: the "Kernkörper" in the cleavage cells arises as "eine Anhäufung von Chromatin, die sich zu einer Kugel abrundet. In ihr taucht bald eine polygonale Felderung als Ausdruck einer schon ganz früh beginnenden wabigen Struktur des Kernkörpers auf." The "Kernkörper" increases in size rapidly, attaining its maximum size and staining intensity when the chromatin filament for the next mitosis becomes well marked. From this time on "beginnt sein allmählicher Verfall"; it stains less intensely, owing to the walls of its meshes becoming thinner; the regularity of the latter becomes lost, and granules appear within and between them, while at the same time the "Kernplasma" ["Kernsaft"] stains more deeply: "Während vorher das Kernplasma hell und der Nucleolus dunkel gefärbt erschien, hebt sich jetzt umgekehrt der helle Kernkörper von dem dunklen Kernplasma ab. . . . Immerhin halte ich es für möglich und sogar für wahrscheinlich, dass zu dieser Zeit ein Austausch zwischen dem Kernsaft und der geformten Substanz des Kernes stattfindet, bei welchem vielleicht ein Theil des vorher im Kernkörper niedergelegten Chromatins dem Kernfaden beigefügt wird." Similar nucleolar changes take place in the male and female pronuclei, antecedent to the stage of the first cleavage spindle; in the male pronucleus "man sieht . . . bei dem aus dem Kopf des Samenfadens sich herausbildenden Spermakern im Gerüstwerk den Nucleolus auftauchen." The younger germinal vesicles contain one deeply staining, homogeneous "Kernkörper"; later vacuoles arise in it, so that it eventually evinces an alveolar structure; the time when the nucleolus disappears is quite variable, thus it may sometimes remain when the chromatin filament is perfected: "Dieser kann übrigens auch noch vorhanden sein, wenn die vier Kernschleifen bereits gebildet sind. Das letztere Verhalten möchte man entschieden so denken, dass die Substanz des Kernkörpers von keinerlei Bedeutung für die Ausbildung der chromatischen

Substanz ist. Das oben eingehend besprochene Verhalten der Embryonalkerne liess dagegen eine ganz andere Auffassung zu, obwohl es auch bei diesen allerdings abnormer Weise vorkommt, dass neben den bereits gebildeten Chromosomen (sogar in der angelegten Spindel) der Kernkörper noch vorhanden ist. . . . Was die erwähnten Verschiedenheiten des Verhaltens der Nucleolen in dem Ei- und Embryonalzellen betrifft, so liessen sich diese vielleicht durch die recht verschiedenartige Ausbildung und Funktion der Kerne in den beiderlei Zellen erklären."

Lauterborn ('95a), nuclear division of *Ceratium hirundinella:* from one to four oval nucleoli are present and are frequently apposed to the nuclear membrane. One nucleolus is still present in the spirem stage (the mitosis advances no further than this); but he was unable to decide whether this nucleolus divides into two.

Lauterborn ('95b), *Multicilia :* each nucleus contains a relatively large nucleolus, which frequently shows a "netzigwabige" structure.

Macallum ('95) concludes that less iron is contained in the nucleolus than in the chromatin, as is shown by its lighter stain with haematoxylin. Nucleoli "are always attached to the chromatin network, and sometimes there appears about them a membrane derived from, and continuous with, the fibrils with which they are connected." In a nucleus of a gland cell from the kidney or liver of *Necturus* "which is passing into the mitotic phase, the nucleolar body disappears, apparently by solution into the chromatin threads, for in the nucleus of a renal cell, in which the meridional disposition of the chromatin filaments obtained preparatory to the formation of the loops, I saw, attached to one of the filaments and partly embraced by its substance, what appeared to be the remains of such a body." The nucleoli of the amphibian ovum are derived from the chromatin of the nuclear reticulum. In support of his previous observations ('91) he adds, "that the iron in the cytoplasm of the ovum makes its appearance only after the solution of the peripheral nucleoli commences." In plant cells (*Erythronium*) there are at least three kinds of nucleoli : the first stain intensely with eosin ; the second are composed of chromatin ;

and the third kind, which occur in the embryo sac, "are not present in the mitotic nucleus, but in the retrogressive stage [metaphase] they appear on the course of the filaments as spherical elements enclosing one or more refracting corpuscles and containing but a small amount of iron, which, however, in later stages . . . is more abundant. These nucleoli are eventually formed chiefly of chromatin, and in stained preparations appear to contain nearly all the chromatin of the nucleus. When mitosis again commences the filament forms at their expense, the increase in size of the filament keeping pace, apparently, with the decrease in the quantity of chromatin which the nucleoli contain. Finally, before their disappearance, when they contain but a minimal quantity of iron, they take the eosin stain deeply. All these forms of nucleoli take up safranin from solutions as readily as do the chromatin elements in the same nuclei, and they hold the stain as tenaciously when they are washed with alcohol. They are in this respect different from the eosinophilous nucleoli in the animal cell, which appear to be unrepresented in the vegetable cell." In *Spirogyra* and *Corallorhiza* "the greater portion of the chromatin in each nucleus forms a single large spherical element unconnected with the chromatin network." He corroborates Leydig's view of the structure of the chromatin loops in the nuclei of the salivary glands of *Chironomus;* the nucleolus is often vacuolar and amoeboid, and may be transversed several times by the chromatin loop; "the presence of granules and vacuoles . . . appears to indicate that it is physically active, which cannot be postulated of the vast majority of the nucleoli of Vertebrate cells." In *Euglena* the nucleolus stains deeply with eosin (except after fixation in picric acid), but does not stain with safranin; it is "intermediate in composition between the nucleolus of higher animal cells and the chromatin of the nuclear reticulum."

Mead ('95), egg of *Chaetopterus:* "in the second cleavage, as in the first, the nucleoli are dropped out into the cytoplasm in the equatorial plane."

Montgomery ('95) described the various arrangements of the nucleoli ("Chromatinmassen") in the ova of *Stichostemma*

*eilhardi.* "Was diese Chromatinmassen chemisch darstellen, ist mir völlig unklar: vielleicht sind sie als von dem Dotter aufgenommene Nährsubstanzen zu betrachten, oder vielleicht stellen sie Konglomerate mehrerer Kernsubstanzen dar." (In my present paper I have no corrections to make to these previous observations, but add only fuller descriptions of the genesis of these nucleoli.)

Moore ('95), spermatogenesis of *Selachii:* the resting nuclei of the first spermatogenetic period contain each a single large nucleolus, which disappears in the following mitosis. In the subsequent resting stage the nucleolus reappears, and also there appears a smaller "secondary nucleolus" surrounded by a vacuole. The larger one then "takes a position, generally, but not always, in line with the long axis of the archoplasm. . . . These two peculiar forms of nucleoli are always to be found after the transition from the first into the second spermatogenetic period, and throughout all the generations of the latter."

Pflücke ('95), ganglion cells of *Invertebrata:* "Ob . . . die zum Nucleolus tretenden Lininfäserchen mit der Substanz desselben verschmelzen oder jener dem Vereinigungspunkt der Gerüstbälkchen nur aufgelagert ist, muss ich unentschieden lassen. Die Nucleolen erhalten sich hierin komplicirter als die Chromatinkörnchen, und die Möglichkeit, dass der intensiv färbbaren Substanz des Kernkörperchens ein eigenes stützendes Liningerüst zu Grunde liegt, ist nicht ausgeschlossen." Nucleolar vacuoles are normal structures, and are especially abundant in the cells of gasteropods; he followed in life the process of the detachment of smaller vacuoles from a larger one, as well as the process of fusion of two vacuoles. In *Helix* "kommen neben drei bis fünf grösseren Hauptnucleolen mit einem oder mehreren Hohlräumen im Inneren, sehr zahlreiche ganz zerstreut liegende kleinere Nebennucleolen bis zur Grösse eines Chromatinkornes herab vor, denen Vacuolen ganz fehlen und die sich von Chromatinkörnchen nur durch die Färbung unterscheiden." He also observed (cells of gasteropods) the "Kernkörperchenkreis" first described by Eimer, and found that the circle of granules around the nucleolus was connected

with it by linin fibers; but he was unable to decide whether these granules are thickenings of linin fibers, or whether they correspond to "Nucleolen bezw. Nebennucleolen . . . , welche sich vielleicht vom Mutterkörper getrennt haben und durch Wirkung centraler Lebensherde in jener typischen, regelmässigen Stellung verharren."

vom Rath ('95a) studied the maturation of the ovum of *Euchaeta marina:* on Pl. VII he figures a number of various sized, all rather large nucleoli, in the germinal vesicle, at the stage when the chromosomes are longitudinally cleft.

vom Rath ('95b) finds that the secretion of the gland cells of the head in *Anilocra* stains exactly like the nucleoli, and concludes that both substances are probably chemically related. He briefly mentions (footnote, p. 5) having seen double nucleoli in liver cells of molluscs and *Amphibia;* these dumbbell-shaped nucleoli may be either regarded as states of fusion or of division. In liver cells of *Astacus* the nucleolus consists of "zwei verschieden tingirten einander dicht anliegenden Kugeln einer dunklen und einer blassen." There is no relation between centrosomes and nucleolar substance.

Rhumbler ('95) studied the nucleolar relations of *Cyphoderia.* From three to nine "Binnenkörper" lie within the nucleus, the largest nuclei having the smallest number; so that accompanying the increase in size of the nucleus, a gradual fusion of the "Binnenkörper" takes place, though without an appreciable increase in the total volume of their substance.

Sacharoff ('95) concludes that since the eosinophilic granules of the blood have the same appearance as the nucleoli, "und weil diese Kernkörperchen bei dem Herausfallen der Kerne auch herausfallen müssen, um dann unweigerlich von Leukocyten verschlungen zu werden, so ist mit grösster Wahrscheinlichkeit anzunehmen, dass bei Säugern die eosinophilen Granulationen auf dem Wege der Phagocytose von aus Hämatoblasten herausgefallenen Kernkörperchen entstehen." In birds the nuclei do not fall out of the erythrocytes, but the eosinophilic corpuscles are nucleoli which have wandered out of the nucleus; these nucleoli are rod shaped. (Only medical literature is cited in this paper.)

Sala ('95), ovum of *Ascaris:* in the first maturation mitosis the single nucleolus breaks into small pieces of various size, which gradually become scattered throughout the nucleus; then they become smaller and spherical, and come to lie directly under the nuclear membrane. These fragments may possibly stand in a genetic connection with the corpuscles which are subsequently found at each pole of the spindle. And since the latter corpuscles may stand in some connection to a centrosome, "es ist . . . nicht unmöglich, dass eine enge Beziehung besteht zwischen der Auflösung des Nucleolus und dem Auftreten des Centrosoma."

Schloter ('95), gland and liver cells of *Salamandra:* in the nuclei may be distinguished, besides the chromatin and paralinin, red-staining spherical corpuscles, the larger of which are regarded as plasmosomes.

Sobotta ('95), ovum of *Mus:* in contradiction to the view of Holl, the chromosomes are not derived from the nucleoli only, but from the whole chromatic substance of the nucleus.

van der Stricht ('95) observed in the larger ovarial eggs of *Amphioxus* that each contains a large nucleolus with an excentric vacuole; it disappears at the time of formation of the pole spindle.

Vejdovský ('95a) found large, homogeneous nucleoli in the yolk cells of *Prorhynchus hygrophilus*, "die nicht die gewöhnliche kugelige Gestalt bewahren, sondern immer in Theilung begriffen sind. Man findet meist doppelte Kernkörperchen, deren Hälften durch eine ziemlich tiefe Furche von einander getrennt sind und die eine centrale Höhlung erkennen lassen. Nebstdem findet man in Drei- selbst Viertheilung begriffene Kernkörperchen. . . . Ich glaube . . . , dass man es hier mit einer Hypertrophie der normalen Kernkörperchen zu thun hat, welche schliesslich zur Degeneration der Kerne führt"; these nucleoli occupy more than two-thirds of the space within the nucleus. In the ovum the nucleolus is much smaller, and shows a division into two parts (Fig. 89), but here these two parts are not of equal size.

Vejdovský ('95b) found in the egg of *Bothrioplana* a spherical nucleolus, "mit einem Nucleolinus."

Waldeyer ('95), cited by Flemming ('96), regards the nucleoli as morphologically distinct from the chromatin reticulum.

Wheeler ('95) observed in *Myzostoma glabrum* that the nucleolus is large and vacuolated, and after the reduction mitosis, "remains in the cytoplasm as an inert mass, gradually melting away, but not disappearing until about the eight-cell stage, when it may often be found in the largest blastomere."

Wilcox ('95) holds that in the spermatocytes of *Cicada* the nucleoli stand in genetic connection with the centrosomes, and adds, "It is probable that different structures have been called nucleoli by different authors."

*1896.*

Auerbach ('96) studied the spermatogenesis of *Paludina:* the nucleus of the spermatogonium contains a number of large, more or less spherical bodies ("Karyosomen"); each nucleolus (of the resting spermatogonium), after simultaneous staining with acid fuchsine and methylen green, shows a central red portion and a blue peripheral shell. "Es besteht also eine Zeit lang der Nucleolus aus einer erythrophilen Centralmasse und einer kyanophilen Rinde." In the subsequent nuclear division of these cells the nucleoli disappear. "Fest steht nur, dass in dem Netzstadium die Nucleoli als solche verschwinden, und dass ihre Rindensubstanz auf die angegebene Art zu einem Teile des intranukleären Netzwerkes wird, der anfangs noch unterscheidbar ist, dann aber durch Auseinanderrücken der Knotenpunkte sich in dem übrigen Fadennetze verliert." In the spirem stage there are one or two small, spherical, red-staining bodies in the nucleus; he was unable to determine whether these stand in any genetic relation to the nucleoli, which had previously vanished. In the spermioblast (which changes directly into the hair-shaped spermatozoön) a small, red-staining body lies within the nucleus, but subsequently disappears; Auerbach supposes that it wanders out of the nucleus and fuses with the "Nebenkern."

Doflein ('96), maturation of the egg of *Tubularia larynx:* the single large nucleolus is suspended by achromatic fibers in

a clear, structureless space within the nucleus ; at first homogeneous, it later contains from one to five unstaining " Körperchen," which he thinks are not vacuoles, on account of their refractibility. In the amitotic division of those nuclei which degenerate and eventually become absorbed by a definitive egg cell, division of the nucleolus precedes that of the nucleus.

Floderus ('96) studied the maturation and embryonal development of various *Tunicata*. A " Hauptnucleolus " and " Nebennucleoli " are present. The former is homogeneous in only very young cells, and later differentiates into two different substances : (1) a refractive, larger portion, which encloses (2) a less-refractive, paler portion. He considers the small vacuoles of the nucleoli to be " Kunstprodukte," though the large one is normal. " Nicht selten findet man in dieser grossen, allem Anscheine nach mit Flüssigkeit erfüllten Höhlung eine Anzahl fester, lichtbrechender Körnchen, vielleicht Coagulationsprodukte, die wahrscheinlich bei der Fixierung entstanden sind." As a rule there is one, but sometimes two " Nebennucleoli " in most though not all eggs ; these rarely attain half the diameter of the " Hauptnucleolus," and appear in the germinal vesicle shortly before the yolk granules arise in the cytoplasm ; they are similar to, but paler than, the refractive portion of the large nucleolus. The " Nebennucleoli " are absent in *Clavelina ;* they probably arise by gemmation from the " Hauptnucleolus," and he figures to this effect a lobular " Hauptnucleolus." In the cytoplasm of the ova of *Styelopsis* and *Ciona* (but not *Clavelina* and *Corella*) certain spherical " intravitelline Körper " occur, usually one to a cell, and frequently close to the nuclear membrane ; in size and staining reactions these are similar to the " Nebennucleoli," and, following Roule, " sehe ich mich genöthigt, anzunehmen, dass sie von Nebennucleolen herrühren, die aus dem Kern des Eies in den Dotter hinausgewandert sind," thereby supposing that they press out through a preliminarily produced pore in the nuclear membrane, and that the larger intravitelline bodies are probably fused masses of smaller ones. In accord with Henneguy ('93) and Roule he considers the intravitelline bodies not as " Dotterkerne " nor astrospheres, but as atavistic or rudimentary organs, which

together with the nucleoli correspond to the macronucleus of the *Infusoria*.

Gerould ('96), ovarial eggs of *Caudina*: in the youngest ova there are numerous peripheral nucleoli; these increase in size as the nucleus grows, and subsequently each contains a vacuole, but they are always close to the nuclear membrane.

Greenwood ('96), macronucleus of *Carchesium polypinum*: the nucleoli ("protomacrosomes," in distinction to the "protomicrosomes," or chromatin granules) are numerous and vacuolated, and stain like true metazoan nucleoli. They vary in size and form, and are probably amoeboid, though this point could not be determined in the living nucleus, which is first rendered visible by reagents. The vacuoles are fluid accumulations, and arise first in the center of the nucleolus. "No vacuoles surround the macrosomes of *Carchesium* at any time, nor do they ever show general increase of fluidity or swelling such as might accompany the penetration through them of some secretion from without; . . . the deposition of vacuolar fluid is centrifugal; . . . thus the macrosome may become a bladder-like or honey-combed structure, its residual solid (?) forming a well-defined membrane-like investment for fluid contents."

Henneguy ('96) distinguishes true and false nucleoli (the latter being "nœuds du réseau," in the sense of O. Hertwig, '92). He reviews the observations upon nucleoli made by several previous authors.

R. Hertwig ('96), unfecundated ova of echinoderms poisoned with strychnine: the nucleoli vanish within the nucleus as the chromosomes appear. "Meine eigenen Untersuchungen lassen es mir ausgeschlossen erscheinen, dass im Ei der Seeigel Nucleolen und Centrosomen irgend etwas mit einander zu thun haben. . . . Dagegen ergeben sich unzweifelhafte Beziehungen der Nucleoli zur Entwicklung der Chromosomen. . . . Dieses Wechselverhältniss ist nun nicht so zu verstehen, als wäre das gesammte Material der Chromosomen in den Nucleoli enthalten. Dagegen spricht die geringe Masse der Nucleolar-Substanz und ihr verschiedenes Verhalten den üblichen Chromatin-Färbungsmitteln gegenüber. . . . Die Nucleolen können somit den Chromosomen ein zur endgültigen Fertigstellung nothwendiges

Ergänzungsmaterial liefern." "Chromatin-Nucleoli" are such as contain the whole chromatin of the nucleus (*Actinosphaerium*, *Spirogyra*, salivary glands of *Culex*); "solche Kerne zeigen dann ein achromatisches Gerüst und in demselben einen grossen chromatischen Körper, im übrigen Nichts, was man den Nucleoli oder den Chromosomen der Gewebszellen vergleichen könnte. . . . Derartige Nucleoli wären dann nicht, wie mein Bruder annimmt, und auch ich früher geglaubt habe, von den echten Nucleoli als etwas wesentlich Verschiedenes zu unterscheiden; sie würden Nucleoli sein, die ausser der specifischen Nucleolensubstanz noch das Chromatin des Kernes enthalten. . . . Bei der Umwandlung zur Spindel lösen sich Chromatinkörnchen vom Nucleolus ab und treten auf das Kernnetz über, ein Substrat hinterlassend, das man wohl den echten Nucleolen vergleichen muss. Später wird auch dieses aufgelöst."

Korschelt ('96), employing a modification of the Ehrlich-Biondi stain, finds in the spinning glands of caterpillars that the macrosomes stain green and hence consist of chromatin, while the microsomes stain red and so must be regarded as nucleoli (cf. '97).

List ('96) made comparative studies on various nucleoli, principally with a view to their chemical constituents, by applying a new staining method, whereby Berlin blue is produced in the fixed tissues. "Wir sind zu dem Resultate gekommen, dass die Nucleolarsubstanzen nach ihrem chemischen Verhalten 3 verschiedene Gebilde darstellen, von denen jedes wahrscheinlich wieder eine eigene complicirte chemische Zusammensetzung besitzt. Nach der bisherigen Bezeichnungsweise sind zu unterscheiden: Hauptnucleolus, Nebennucleolus und Nucleolus schlechtweg"; the substance of all the nucleoli differs from that of the nuclein (chromatin) proper. "Wir haben gesehen, dass (bei Mytilus und Pristiurus) die Umsetzung des Ferrocyankaliums durch Salzsäure, wodurch Ferrocyanwasserstoffsäure und hieraus durch den Sauerstoff der Luft Berlinerblau entstand, allein genügte, um die Nebennucleolen zu färben. . . . Wenn wir die Reagentien concentrirter anwenden, . . . so tritt in jeder Zelle die Substanz des Nucleolus in Gestalt eines oder

mehrerer blauer Kügelchen hervor. . . . Nach ihrem chemischen Verhalten stehen also Nebennucleolus und Nucleolus einander näher als Haupt- und Nebennucleolen"; he concludes that the "Nucleolus" and the "Nebennucleolus . . . mindestens verschiedene Modificationsstufen des Paranucleins . . . darstellen." *Mytilus* egg: what Lönnberg supposed to be vacuoles within the nucleoli, List holds are "Nebennucleoli," and these alone evince the characteristic Berlin-blue reaction; by afterwards staining the preparation with carmine, "die Masse des Hauptnucleolus, das Nuclein, hatte sich scharf roth gefärbt, die Nebennucleolarsubstanz, das Paranuclein, rein blau." Even in eggs where no yolk was as yet present, both these substances could be demonstrated. The "Hauptnucleolus" represents the greater part of the nucleolus, and is usually single; in it may lie one spherical "Nebennucleolus," or the latter may cover, cap-like, one pole of the former; sometimes "Nebennucleoli" occur in the nuclear cavity, separated from the "Hauptnucleolus"; occasionally there are true vacuoles within the latter. *Pholas* egg: (treatment with iron chloride, nitric acid, then "Ferrocyankaliumlösung") the "Nebennucleolus" is much larger than the "Hauptnucleolus," except in very small ova, where they may be equal in size. The last-named nucleolus may enclose a large, excentric vacuole, or in place of this, a "Nebennucleolus"; in the chromatin network of the nucleus there are small nodules of paranuclein, and sometimes a free "Nebennucleolus." "In älteren Eiern überwiegt bei Weitem der Nebennucleolus den Hauptnucleolus an Masse"; the latter is either apposed to one end of the former, or there may be a large "Nebennucleolus" with a small "Hauptnucleolus" at each end of it. *Pristiurus* egg: in the youngest germinal vesicles the minute nucleoli all lie at the nuclear periphery, the larger ones being central; in larger ova all the nucleoli are placed at the periphery of the nucleus. *Sphaerechinus* egg: the supposed (Häcker, '93b) vacuole of the "Nebennucleolus" is in reality the "Hauptnucleolus": "Jedoch weichen meine Resultate von denen Häcker's darin principiell ab, dass eben festgestellt werden konnte, dass das, was H. Hauptnucleolus nennt, wie ein Nebennucleolus reagirt, und die Vacuole

wie ein Hauptnucleolus." With the three staining methods employed (all used on material fixed with corrosive sublimate), only the "Nebennucleolus" is plainly stained, "nicht aber der Nucleolus schlechthin, wie er in jeder Zelle vorkommt." By treatment for half an hour with a drop of .5 % iron chloride solution, then stained by the Berlin-blue reaction, in each somatic cell the nucleolar substance appears in the form of bluish-green spherules. "Im Mollusken- wie im Vertebratengewebe hatte jede Zelle einen rundlichen Nucleolus; in secernirenden Zellen, z. B. Darmzellen, traten 2 oder 3 auf, oder Grössenunterschiede, wie z. B. der Nucleolus in der Leberzelle von Mytilus durch seine Grösse auffällt."

Michel ('96), ova of *Nephthys* and *Spiophanes:* each double nucleolus consists of (1) a darker, more granular, portion, which in *Spiophanes* contains either a small granule or a vacuole (he is undecided which it is), and in *Nephthys* is vacuolated; and (2) of a clearer, refractive, unstaining portion. In *Nephthys* there are usually two double nucleoli, "la substance colorable recouvrant plus ou moins complètement la masse claire comme d'une calotte"; but other states were also found: "trois nucléoles doubles, une sphère claire entre deux parties sombres presque à l'opposé; inversement, une partie sombre et deux sphères claires presque opposées, nucléoles plus composés avec plusieurs sphères claires et même comme spumeux, sphérules claires libres en plus de celles des nucléoles doubles jusqu'à une douzaine. . . . Les masses claires, avec leur aspect, leur forme sphérique et leur déformation temporaire par la pression, leur variation de taille suivant les conditions osmotiques, l'épaississement de leur paroi par réduction de volume, apparaissent comme des vésicules à contenu liquide spécial," while the colorable portions are composed of pyrenin, and hence are true nucleoli (the pyrenin proved "par l'absence de gonflement par l'eau et par le gonflement par les acides, par l'insolubilité dans le sulfate de cuivre ou le ferrocyanure de potassium. . . . l'aspect des vésicules et leur disposition dans les nucléoles ou à l'état libre . . . portent à croire à des vacuoles à contenu spécial formées dans le nucléole et finalement éliminées").

Morgan ('96) studied Echinoderm eggs placed in artificial

media: immature ova of *Sphaerechinus*, placed in sea water to which 1.5 gr. NaCl had been added, show artefacts in the nucleolus: "Each [body] consists of an outer darker shell, which is filled with a clear fluid, and the center of each sphere is occupied by a small black granule"; several of these structures are usually found on each section through the nucleolus. (For previous descriptions of somewhat similar productions, *cf.* Ransom ('67), Leydig ('88), and O. Schultze ('87). The upper of the two figures numbered "24" in Morgan's plate should be "23," since it refers to the nucleolus.)

Rohde ('96), ganglion cells of *Doris* and *Pleurobranchus*: the nucleoli wander out of the nucleus and finally into the neuroglia, and there acquiring an envelope (derived from the neuroglia) form new cells. [Judging from his figures, however, these supposed nucleoli would seem to be myelin drops.]

Wagner ('96a), spermatogenesis of *Arachnids*: "Bei der ersten Spermatocytentheilung theilt sich der Nucleolus entweder in der Ebene der Aequatorialplatte mit den Chromosomen zusammen, oder ausserhalb derselben neben einem der Spindelpole. Im letzteren Falle tritt er nach dem Verschwinden der Kernhülle . . . aus dem Kerne heraus."

Wheeler ('96) gives no description of the nucleoli in the text, but he figures several stages of the development in eggs of *Myzostoma* (Figs. 9, 10–15, *M. cirriferum*; Figs. 23, 52–54, 56, *M. glabrum*). In *M. cirriferum* (Figs. 12–15) is figured, in addition to the single large nucleolus, also one smaller nucleolus.

E. B. Wilson ('96) states of the true nucleoli or plasmosomes: "There is strong evidence that the true nucleoli are relatively passive bodies that represent accumulations of reserve-substance or by-products, and play no direct part in the nuclear activity." In germinal vesicles he assumes that the "principal nucleolus" is chemically different from the nucleoli of somatic cells; but that the "accessory nucleoli" of the former correspond to the nucleoli of the latter. He concludes that "we can hardly doubt the conclusion of Häcker, that the nucleoli of the germ-cells are accumulations of by-products of the nuclear action, derived from the chromatin either by direct transformation of its substance, or as chemical cleavage-products or secretions."

*1897.*

Toyama, cited by R. Hertwig ('96), holds that the nucleoli become centrosomes in the spermatogenesis of *Bombyx*.

Van Bambeke ('97a), ovocyte of *Pholcus:* there is usually a single large nucleolus, rarely also accessory ones ; the nucleolus is vacuolated, "les vacuoles . . . faisant fréquemment saillie à sa [tache germinative] surface ; dans certains vacuoles, on découvre des granules safraninophiles." At a later stage the nucleolus retains much the same appearance, "mais fréquemment le contour net, safraninophile, qui la délimitait, a disparu en tout ou en partie, et l'on remarque parfois une solution decontinuité au niveau de laquelle le contenu de la tache s'épanche dans le reste du contenu nucléaire. Cette sorte d'évacuation ne doit pas être confondue avec la rupture de vacuoles nucléolaires, laquelle peut s'observer à tous les stades." ('97b, the same, with figures.)

Bouin ('97), giant spermatogonia of *Cavia:* the accessory part ("corps juxtanucléolaire") of the double nucleolus stains red in safranine and blue in haematoxylin (in opposition to Hermann), though less deeply than the spherical portion of the nucleolus, and is sometimes hemispherical in form. This part is single, and appears to consist of a mass of very fine granules. In degenerating cells, "les uns nous montrent deux nucléoles flanqués chacun d'une ou de plusieurs petites masses hémisphériques, réfringentes et teintées en rose pâle ; lors des mouvements intranucléaires, les corps juxtanucléolaires contractent des rapports plus intimes avec les vrais nucléoles, deviennent plus réfringents et moins colorables, s'accolent à leur substance, se divisent à leur suite, et les accompagnent dans leurs migrations. Après plusieurs divisions répétées, ces noyaux contiennent un certain nombre de nucléoles, cinq ou six généralement." In the process of formation of the cells of Sertolli the nucleoli fuse successively with one another.

Braem ('97), *Plumatella:* in the egg of .013 mm. diameter the nucleolus contains one to four vacuoles: "Sie sind allem Anschein nach Flüssigkeitsbläschen, welche im Nucleolus auftreten und auf dem Höhepunkt ihrer Entwickelung an die

Peripherie rücken, um da ihren Inhalt nach aussen zu entleeren." The nucleolus becomes ovoid, and its substance paler at its smaller end; the vacuoles are usually, but not always, at the paler end. "Zuweilen ist der Gegensatz der beiden Nucleolus-Hälften lediglich in der verschiedenen Färbbarkeit derselben ausgesprochen. In anderen Fällen wird er durch eine Einschnürung bezeichnet, die den Nucleolus in einen grösseren, dunkeln und einen kleineren, hellen Abschnitt zerlegt. . . . Die Einschnürung kann nun zu einer völligen Abschnürung führen, so dass der Nucleolus doppelt erscheint und von zwei neben einander liegenden Kugeln gebildet wird, oder bei gegenseitiger Entfernung der Theilstücke in zwei räumlich getrennte Nucleoli zerfällt. . . . Selten ist der Nucleolus dreitheilig . . . , wo das mittelste Stück dunkler ist als die beiden seitlichen. . . . Dies lässt vermuthen, dass der Keimfleck im Stande ist, unabhängig vom Wachsthum des Eies seine Gestalt zu verändern, und dass die Zweitheiligkeit auf der Bildung eines pseudopodienartigen Fortsatzes beruht, der sich bald mehr, bald weniger deutlich vom Hauptkörper abgegliedert und auch hinsichtlich seiner Substanz bald mehr, bald weniger von demselben verschieden ist."

De Bruyne ('97), double cells of the ovarian follicle of *Nepa*, *Periplaneta*, *Meconema*, and *Aeschna:* in the amitotic division of the nucleus the nucleolus divides first. (Since the cytoplasm does not divide, each such cell finally receives two nuclei.)

Carnoy and Lebrun ('97a) (an abstract of this paper may also be found in the *American Naturalist* for July, 1897). This contribution deals particularly with the relations of the nucleoli in the growth period of the ovum of *Salamandra* and *Pleurodeles*. In the youngest nuclei observed there is a nuclein filament, but no nucleoli; the first nucleoli arise as buds from the filament, and these are termed "nucléoles primitifs." Then the nuclear filament becomes changed into an amorphous magma, composed of irregular granules, and the latter then subsequently disappear, so that all trace of the original filament becomes lost. All further changes within the nucleus are of nucleolar character. From the "nucléoles primitifs" are derived the "nucléoles secondaires" which "sont dûs à des

associations de granules provenant de la désagrégation de l'élément nucléinien" ; and then follow the "nucléoles tertiaires," which differ from the nucleoli of the preceding two generations in that they do not come from degenerating granules of preceding generations, but are detached from them in the form of spherules. Each nucleolus of each generation arises, increases in size, becomes more complex in structure, and then passes through a polymorphic "figure de résolution" ; the form of these figures varies according to the particular generation, and also according to particular ova. The greater part of the "figure de résolution" then disappears, except a few granules which serve as the starting point for the next generation ; that portion of the substance which disappears serves as nourishment for the egg. So all the generations of the secondary and tertiary nucleoli arise "à l'aide des produits de la résolution antérieure." After each "résolution" new nucleoli arise, and the number of these generations is large, continuing through a length of three years. The number of primary nucleoli is usually from two to six ; of secondary, from 400 to 500 ; of tertiary, from 500 to 1000 ; the number varying in different ova. Fusions of nucleoli are of normal occurrence : "cette attraction des masses nucléiniens rappelle à l'esprit ce qui se passe au sein de l'œuf entre les noyaux de conjugaison." In the radiation exerted by each nucleolus upon the surrounding caryoplasm "nous voyons . . . la confirmation d'une thèse soutenue dans la 'Cytodiérèse,' à savoir : que c'est sous l'influence du noyau que se forment les asters de division." The chromatin filament does not reappear, but there is a "grand nombre de générations nucléolaires et filamenteuses qui naîtront et disparaîtront tour à tour, l'une après l'autre, jusqu'à l'époque des globules polaires." The authors necessarily regard all the previous observations on the amphibian ovum as erroneous. General conclusions for all kinds of cells, based in part on previous observations : there may be distinguished "nucléoles plasmatiques," "nucléoles nucléiniens," and "nucléoles mixtes" ("qui sont rare"). Plasmatic nucleoli consist of at least two substances, "une plastine et une globuline digestible." All nucleoli, "lorsque leur formation est achevée . . . représentent

la totalité de l'élément filamenteux d'un noyau ordinaire ; . . . dans bien de cas — aujourd'hui nous pourrions peut-être dire dans tous — on constate dans ces corps la présence d'un véritable appareil filamenteux, tortillé sur lui-même, comme dans un noyau ordinaire, et présentant les mêmes proprietés que dans ces derniers. C'est que l'on voit surtout dans les nucléoles-noyaux, c'est-à-dire dans les nucléoles nucléiniens uniques, qui ont absorbé tout l'élément filamenteux primitif." All nucleoli develop from the chromatin filament ; and chromosomes are derived from "nucléoles noyaux." The chemistry of nucleoli is also considered.

Carnoy and Lebrun ('97b), fecundation of the ovum of *Ascaris megalocephala:* the centrosomes are "nucléoles plasmatiques ou achromatiques" which have left the nucleus at the commencement of mitosis ; one is derived from the male, the other from the female, nucleus. They totally disappear after mitosis, and neither reënter the nuclei nor divide to produce the centrosomes of the subsequent division.

Cunningham ('97) : "There are indications in the ova of the turbot that the substance of the nucleoli is absorbed into the central fibrils to form the chromosomes of the polar mitoses, but the actual formation of these chromosomes was not followed."

v. Erlanger ('97), a brief mention of certain recent views upon the nucleolus : "Als echte Nucleolen waren allein solche Körper zu bezeichnen, welche sich durch ihr Verhalten gegen Chemikalien . . . scharf von dem Chromatin unterscheiden. . . . Vorderhand bleibt also die Bedeutung der echten Nucleolen rätselhaft, falls man diese Gebilde nicht mit Häcker als eine Sekretion des Kernes beurteilen will." They bear no relation to centrosomes.

Fauvel ('97), ovogenesis of *Ampharete :* ovarial ova of 30$\mu$ diameter, and at this stage only, contain two nucleoli. "On rencontre toutes les modifications : nucléole simple, nucléole étranglé par le milieu, deux nucléoles accolés, et enfin deux nucléoles bien nettement séparés. . . . Nous n'en avons jamais rencontré deux dans l'œuf mûr, ni dans l'œuf non détaché de l'ovaire." The nearly mature ovum contains one

large nucleolus, with a large vacuole ; he believes that subsequent to the two-nucleolus stage one of the nucleoli is extruded from the nucleus. Two nucleoli were observed also in the ova of *Amphicteis*, *Sanytha*, and *Melinna*.

Flemming ('97) recurs to the controversy between Korschelt ('96) and Meves ('97), and agrees with Meves that the macrosomes are nucleoli, and the microsomes chromatin granules. He also mentions the following observation on the ovum of *Ascidia canina :* here there is one "Nucleolus" and one much smaller "Kernkörper" ; "beobachtet man ihn [Kernkörper] am lebend entnommenen Ei, so findet man ihn so gut wie stets in Molekulärbewegung, und zwar oft in recht grossen Exkursionen."

Häcker ('97a) ('96 is a preliminary communication), cleavage stages of *Cyclops brevicornis*. This paper deals particularly with the "intrasphärale," "extranucleäre," or "Aussen-Körnchen (Ektosomen)" found in certain of the astrospheres of the cleaving ovum. These ectosomes are small spherical bodies of various size, which stain like the nucleoli, but somewhat more intensely. In the resting stage of the cell there are several nucleoli in the nucleus, and no ectosomes outside of it ; when the nucleus enters on the aster stage, the nucleoli have disappeared and ectosomes are present in one of the astrospheres, at first at the base of, subsequently on the whole periphery of, the latter ; towards the close of the metakinesis there appear in the place of the ectosomes larger clumps of red-staining substance. He concludes : "So glaube ich es denn mit Sicherheit aussprechen zu dürfen, dass diese gröberen Brocken auch genetisch mit den Körnchen [Ektosomen] zusammenhängen, sei es, dass sie direkte Umwandlungsprodukte derselben, sei es, dass sie Neubildungen sind, welche dem nämlichen Process ihre Entstehung verdanken, aber in Folge der während der Theilung eintretenden Zustandsänderungen der Zelle eine etwas andere Beschaffenheit, einen anderen Aggregatzustand angenommen haben. Wie ich gleich hier hinzufügen möchte, verschwindet die Erscheinung, sowohl im Zweizellenstadium als in den späteren Stadien, während der eigentlichen Ruhepause vollständig, indem vermuthlich jene Massen einer Resorbtion

oder chemischen Umwandlung anheimfallen." In only one astrosphere of only one cell in each of the following cleavage generations this process is repeated, and the line of these particular cells ("Körnchen-Zellen") constitutes the line of development of the sexual cells ; but the ectosomes are present in these particular cells only during mitosis, and in the resting stages are absent, while nucleoli occur in the nuclei ; this process was observed from the first through the ninth cleavage stages. He concludes that in each generation there is a production *de novo* and a subsequent solution ("Auflösung") of the ectosomes. The first appearance of the latter coincides in point of time approximately with the disappearance of the nucleolar substance in the nucleus ; from this and certain other factors he concludes : "So . . . würde also der Annahme kaum etwas im Wege stehen, dass die zu Beginn der Mitose noch vorhandenen oder neugebildeten Nucleolen aus dem Kernraum in der Richtung der *einen* Spähre auswandern und sich hier in die Aussenkörnchen umwandeln. . . . Für die Kerne der Körnchenzellen ist dann allerdings, in Gegensatz zu den übrigen Embryonal-Elementen, eine besonders reichliche Produktion der Nucleolarsubstanz und demnach eine besonders intensive vegetative Thätigkeit [*cf.* '95] anzunehmen." The explanation for the arrangement of the ectosomes in only one of the astrospheres he finds in the assumption "dass die beiden Centrosomen einen verschiedenen (vielleicht einen verschieden 'kräftigen') Einfluss auf das umgebende Plasma, beziehungsweise auf die beweglichen Inhaltskörper desselben ausüben."

Häcker ('97b) finds that in germ cells of both animals and plants there is to be noted "das Auftreten eines einzigen, vacuolenhaltigen, dunkel tingierbaren "Hauptnucleolus" in den jüngeren Stadien, das Hinzutreten von blässeren adventiven oder "Neben-Nucleolen" in einer früheren oder späteren Phase." Nucleolar substance arises during one or several stages of nuclear activity as a by-product of metabolism, possibly also as chromatin substance which has become structureless and chemically changed; and, finally when the nucleus begins to divide, is removed out of the latter. He confirms Wheeler's ('96) observations on the ovum of *Myzostoma*, that the nucleolus

wanders out of the nucleus into the cytoplasm, where it slowly decreases in size.

Hermann ('97) figures (Fig. 20) a spermatogonium nucleus of Scyllium containing a single and a double nucleolus ; the latter consists of two apposed spheres, which differ chemically and dimensionally.

Korschelt ('97) maintains his previous opinion ('96) of the chromatin nature of the macrosomes of the nuclei in the spinning glands of caterpillars, in answer to the criticism of Meves ('97) (reviewed immediately below). Korschelt employed the Ehrlich-Biondi stain with increased strength of the methyl green, and thereby obtained a coloration of the macrosomes and microsomes the very opposite of that procured by Meves. "Ob man überhaupt achromatische, chromatische Substanz und Nucleolen in allen Kernen so scharf auseinanderhalten kann, wie dies vielfach geschieht, ist mir höchst zweifelhaft. Wenn man in verschiedenen Zuständen der Kerne Nucleolen auftreten und wieder verschwinden sieht, wird man annehmen müssen, dass sie sich aus den sogenannten achromatischen oder chromatischen Substanzen des Kerns, vielleicht aus beiden herausbilden. So können sich möglicher Weise auch die von mir als Makrosomen bezeichneten Theile in Nucleolen umbilden und das von Meves angegebene Auftreten von Vacuolen in ihnen würde damit seine Erklärung finden."

Meves ('97) contends that the microsomes in the spinning glands of caterpillars, which Korschelt regarded ('96) as lanthanin granules, are chromatin ; and what Korschelt regarded as chromatin granules (*i.e.*, the macrosomes) are nucleoli. Meves employed the usual formula of the Ehrlich-Biondi stain (Heidenhain's receipt), and finding that the macrosomes thereby become stained red, concludes from this reaction their chromatin nature.

Stauffacher ('97) finds in the aster stage of the mitosis of one of the pronephral cells of *Cyclas*, that the nucleolus still persists intact in apposition to the spindle fibers.

Wheeler ('97), maturation of the ovum of *Myzostoma :* this object, previously described by the author ('95), is here more fully described with the addition of figures. A remarkable mode of formation of nucleoli in the pronuclei is described ;

each "chromosome" consists of "two granules, at first of the same size [which] grow very unequally, so that one is often considerably larger than the other. Hereupon some, but not all, of these granules break down to form irregular strings of minute karyomicrosomes which are distributed along the fibers of the achromatic reticulum. . . . The large chromatin granules which do not break down become the nucleoli of the pronuclei. I am unable to state positively that in each Diplococcus-shaped chromosome one of the granules breaks down to form a chain of minute karyosomes while the other persists as a nucleolus, but I am very strongly inclined to believe that this is the case." These nucleoli are cast out into the cytoplasm when the first cleavage spindle is formed, and there rapidly dissolve. Wheeler accepts "Häcker's view of the secretory nature of the nucleolus, at least so far as the germinal vesicle is concerned."

Bancroft ('98), germinal vesicle of *Distaplia:* the nucleolus "does not form the stellate body found in the old ova, as Davidoff maintained, but is found within this body, which is itself the remains of the germinal vesicle. The nucleolus at this stage is quite complex, consisting of a homogeneous cortex, an excentric finely granular medulla, and within the latter several very highly refractive bodies, the largest of which may have a granular appearance. During the greater part of the growing period these refractive bodies are the only substance in the germinal vesicle that takes the chromatin stain with a methyl green and acid fuchsine combination."

*1898.*

Kostanecki ('98) confirms the observations of Wheeler ('95, '97) in regard to the casting out of the nucleolus into the cytoplasm, in the maturation of the ovum of *Myzostoma.*

## *B.* Botanical Literature.

Schleiden ('38) is the discoverer of the nucleolus in plants, but he gives it no name: "einen kleinen, sich scharf abgrenzenden Körper, der, nach dem Schatten zu urtheilen, einen

dicken Ring oder ein dickwandiges hohles Kügelchen darzustellen scheint"; while in other cases it may be a simple spot, or may be wholly absent. "Aus meinen Beobachtungen an allen Pflanzen, die eine vollständige Verfolgung des ganzen Bildungsprocesses erlaubten, geht hervor, dass dieser kleine Körper selbst früher sich bildet, als der Cytoblast [Nucleus]."

Macfarlane ('81) examined various plant cells, in all of which he found one or several bodies ("nucleolo-nuclei") within the nucleolus. The nucleolus of *Spirogyra* has a distinct membrane, which disappears at the period of the nucleolar division; the karyokinesis results in the formation of a "nuclear barrel," at each end of which is a mass of nucleoplasm, these two masses being connected by fibers with the nucleolus which lies between them. The nucleolus then divides, preceded by a division of the nucleolo-nucleus, so that each daughter-nucleolus receives a daughter nucleolo-nucleus, and the daughter-nucleoli then wander apart to the nearest masses of nucleoplasm, "as they retreat from each other they drive the polar masses before them, thereby elongating the nuclear barrel. . . . The nucleoli at length advance to the polar masses and bury themselves in the nucleoplasm of these." From these and numerous other observations, Macfarlane concludes: "that the nucleolus, or more probably the nucleolo-nucleus, is the center of germinal activity, and that as we pass outwards to the periphery of the cell, this reproductive activity becomes less and less. In no other way, to my mind, can the number of nucleoli and nucleolo-nuclei at different ages in the cells of any plant be explained."

Strasburger ('82a) gives reviews of previous observations on the chemical constituency of nucleoli.

Strasburger ('82b) studied nuclear division in various plant cells (*Fritillaria, Lilium, Hemerocallis, Tradescantia, Galanthus, Dicotyledons*). "Pollenmutterzelle" of *Fritillaria:* between the nucleus and its membrane collects a homogeneous, refractive, lens-shaped mass of substance; "sie geht nicht unmittelbar aus den Kernkörperchen hervor, die ja schon auf vorausgehenden Stadien verschwunden waren, vielmehr repräsentirt sie, allem Anschein nach, ein Secret"; this body he terms "Secretkörperchen." At first it stains deeply with methylen green; but

subsequently it ceases to stain, vacuoles arise in it, and it decreases in size, until at the time of the spindle formation it disappears. "Sie [Secretkörperchen] treten erst auf, nachdem das Kernkörperchen oder die Kernkörperchen in dem Fadenknäuel des Kerns Aufnahme gefunden. Ihre Entwicklungsgeschichte unterscheidet sich auch von derjenigen echter Nucleolen, denn sie treten nicht im Verlauf der Fadenwindungen auf, vielmehr ausserhalb derselben, stets an der Wand der Zelle. Ausgeschlossen ist ja nicht, dass in der so ausgesonderten Substanz die Substanz früher Kernkörperchen vertreten sei, aber erweisen lässt sich dies nicht." So he concludes that before the mitosis of the spores and "Pollenmutterzellen" a certain change occurs in the nucleoplasma, in connection with the formation of the "Secretkörperchen." The nucleoli of many plant cells contain vacuoles. In the embryo sac of *Galanthus* a division of the large nucleolus takes place, which division is probably passive, caused merely by the tension of the cytoplasm. Gradations are to be found between the nucleoplasmic-microsomic substance and the substance of the nucleoli: "ob die Nucleolen-Substanz trotzdem nur eine Modification der Microsomen-Substanz sei und aus dieser hervorgehe, will ich dahingestellt bleiben lassen. Wahrscheinlich ist mir aber das letztere, wenn ich bedenke, dass bei Eintritt in die Theilungsvorgänge selbst die stark modificirte Nucleolen-Substanz in das Kerngerüst findet und sich in demselben nicht anders als wie die Mikrosomen-Substanz verhält. Man könnte die Nucleolen-Substanz vielleicht als einen Reservestoff des Zellkerns auffassen, als eine momentan ausser Aktion gesetzte Substanz."

Tangl ('82) studied the nuclear division of three species of plants. *Hemerocallis fulva*, flower buds: the "Pollenmutterzelle" contains at first three or five nucleoli, which are homogeneous. "Mit fortschreitender Entwicklung der Mutterzellen verringert sich die Anzahl der Nucleolen," until only one is to be found; this one is always peripheral in position, never in contact with the central "Körnermasse." Later, vacuoles appear in the nucleolus (he believes these to be the results of reagents), and while it still stains with carmine it no longer does with acidified

methylen green. In mitosis, when the nucleus is uninucleolar, the substance of this nucleolus becomes dissolved in the nucleus; when multinucleolar, however, one of the nucleoli may pass out into the cytoplasm. *Hesperus*, "Pollenmutterzelle": here there is one nucleolus, which stains with methylen green, as does the chromatic filament, and disappears in mitosis. *Pisium*, same cells: here there is one hat-shaped nucleolus, which stands in no connection with the "Fadenknäuel"; "Sehr eigenthümlich ist das Verhalten des Nucleolus in den die Kerntheilung vorbereitenden Stadien. Anfänglich besteht derselbe aus homogener, stark lichtbrechender Substanz. Später sind am Nucleolus eine dichte äussere und eine innere, bedeutend schwächer lichtbrechende Schichte unterscheidbar. Endlich findet man Stadien, auf denen neben dem noch unveränderten Fadenknäuel ein sehr schwach lichtbrechender Körper gefunden wird, dessen Umrisse vollkommen demjenigen des ursprünglichen Nucleolus entsprechen"; finally even this disappears.

Zacharias ('82) studied the epidermis cells of *Phajus*, and concludes that the nucleoli (one or two in number) consist of plastin. They do not dissolve in distilled water; swell with .1% nitric acid; do not stain with methylen green; and dissolve in weak "Kalilaugelösung."

Heuser ('84) studied the mitoses in the embryo sac of *Fritillaria imperialis*. In the resting nucleus there are from five to nine nucleoli: "Dieselben sind intensiv gefärbt und stehen in deutlich wahrnehmbaren Zusammenhang mit dem Nucleo-Hyaloplasma." In the prophase of the mitosis they lose their staining power and apply themselves to the chromatin threads. He considers them, with Strasburger, "als Reserve-Behälter der Kernsubstanz" (using the term "Kernsubstanz" as equivalent to "Chromatin"); their ground substance consists of plastin, permeated with chromatin. In *Fritillaria*, as well as in *Galanthus* and *Leucojum*, "fliesst das gesammte Kernkörperchen in die Kernelemente über, während in anderen Fällen ein Ueberschuss an Plastin als 'Secretkörper' ausgeschieden werden mag."

Strasburger ('84), nuclei in the embryo sac of *Fritillaria*: in the spirem stage the large nucleoli disappear, "wobei sich um

dieselben der Kernsaft wieder zu färben beginnt." He concludes that the nucleoli are not immediately taken up into the chromatic thread, but dissolve in the caryolymph; "auch ist hiermit wohl sicher der Nachweis gegeben, dass sie nicht identisch mit den Mikrosomen sein können." The nucleoli arise in the meshes of the chromatin network. Strasburger agrees with Flemming that they represent a substance distinct from the chromatin and nuclear sap, but does not consider it to be a living substance, but rather a reserve stuff.

Guignard ('85) investigated nuclear division in several species of plants. *Lilium*, young embryo sac: the nucleus usually contains a single nucleolus, which is very large, finely granular in structure, and situated excentrically between the strands of the chromatin network; with the double stain, methylen green and fuchsine, it stains red, while the chromatin stains green. At the time of the longitudinal division of the chromatin filament, the nucleolus commences to stain less intensely, vacuoles arise in it, and it finally fragments into small pieces which subsequently disappear; the fine granules appearing in the nuclear sap at this time are not derivatives of the nucleolus, but originate from the cytoplasm when the nuclear membrane vanishes. "Dans le *Lilium* . . . rien ne fournit la preuve d'un apport direct effectué dans la formation du fuseau par le nucléole, dont la substance se dissout dans le suc nucléolaire, pour s'incorporer et se mélanger, . . . aux autres éléments figurés qui contiennent la chromatine." In each daughter-nucleolus there are several nucleoli of unequal size; these disappear also in the subsequent mitosis. *Clematis*, embryo sac: the nucleoli in karyokinesis gradually decrease in size, and it seems "comme si la plus grande partie de leur substance était absorbée par les segments [chromatiques]." *Northoscordum:* here there are several large nucleoli which disappear when the spindle is produced, their substance being possibly incorporated in the chromosomes. In the metaphasic spirem they reappear in contact with the chromatin: "leur aspect général fait supposer qu'ils naissent là où on les aperçoit dans les jeunes noyaux . . . il est à croire que les nucléoles tirent une partie de leur substance, tout ou moins, du filament nucléaire auparavant

homogène. Ils se nourrissent ensuite dans le suc nucléaire. . . . Les nucléoles peuvent être considérés comme une substance de réserve que se sépare à un moment donné de la charpente nucléaire pour être reprise par elle ultérieurement"; he assumes that Strasburger's "corpuscule du sécrétion" is a true nucleolus. "Dans le *Lilium* et dans l'autres plantes, les noyaux filles n'offrent pas de nucléole avant d'entrer en division; en outre, leur aspect général au début du phénomène est bien different de celui du noyau mère. . . . Le fait qu'ils se séparent du filament dès que le noyau . . . arrive à l'état de repos, pour être repris par lui aux premiers stades de la division, permet de les considérer, avec M. Strasburger, comme une sorte de réserve."

Macfarlane ('85) studied nuclear division in *Chara fragilis* (fixation with osmic acid): the nucleus of the apical cell contains one nucleolus, in which lies an "endonucleolus" (a term here substituted for his earlier term "nucleolo-nucleus"). At the commencement of all cell divisions this part of the nucleolus first divides, then the nucleolus, last of all the nucleus. After this division of the apical cell a nodal and internodal cell are produced, and the former "continues to divide regularly, forming cells each with one nucleus and nucleolus. In the internodal complete cell division is henceforward absolutely arrested: but the earlier steps are taken; for while the nodal cell has divided into three or four, the nucleolus of the internodal has divided and redivided, so that four nucleoli are present in the nucleus of it. The internodal cell then increases rapidly in length, the four nucleoli meanwhile continuing to proliferate, so that in internodal cells, such as in the third removed from the apex, we soon get a large nucleus with many little dark nucleoli. The nucleus then divides in the simple manner figured by Johow, so that in the fourth internodal cell there may be two nuclei, each with many nucleoli, in the fifth, three or four nuclei, and so on, so that the internodal cells soon become multinuclear, and their nuclei multinucleolar." The cortical nodal cells do not divide further, but "their nucleoli follow the example of that of the internode . . . the consequence being that the cortical nodal, and soon after the cortical

internodal cells, become multinucleolar"; the nodal leaf cells proceed in the same way. From these observations Macfarlane concludes: "in every active embryonic cell one nucleolus only is present in the resting state"; in some cases a fluid globule is present in the nucleolus, and this probably represents a "degradation of the endonucleolus." "The nucleolus, or more probably the nucleolo-nucleus, is the center of germinal activity, and that as we pass outwards to the periphery of the cell, this reproductive activity becomes less and less. . . . The result is that in all plants thus examined, after cell division has ceased, continued division of the cell contents from the endonucleolus outwards goes on. . . . I venture, therefore, to regard it as a general principle that after cell formation has ceased, the cell contents (especially the endonucleolus and nucleolus) persist in their activity for a shorter or longer period; . . . the most exalted type of cell is one with abundant protoplasm containing a single nucleus, nucleolus, and endonucleolus; . . . a cell with vacuolated protoplasm, one nucleus and two to four nucleoli is less exalted, while the multinuclear state is the most degraded form of cell."

Zacharias (**'85**) gives critical reviews of numerous preceding papers on nucleoli, besides observations of his own on various cells of plants. *Galanthus nivalis*, cells of the inner layer of the "Fruchtknotenwand": the single nucleolus is about $\frac{3}{10}$ the size of the nucleus; examined in water it is homogeneous; after the action of absolute alcohol it appears to be composed of granules of various indices of refraction. Bast cells of *Cucurbita pepo*: the nucleoli, when stained with "Blutlaugensalz-Eisenchlorid," become very intensely colored, while the remaining nuclear substance stains only faintly. In the cells of *Spirogyra* and of the asci of Lichens he finds that there are no "nucléoles-noyaux," such as Carnoy described. "Alle Autoren stimmen gegenwärtig darin überein, dass die Nucleolen bei der Kerntheilung verschwinden." In opposition to Strasburger he contends that during the mitosis the dissolved nucleolar substance might as probably enter into the formation of the spindle fibers as of the chromosomes. In *Chara* (observed living) each nucleus contains one large nucleolus, with vacuoles: "Naht

die Kerntheilung heran, so verliert der Nucleolus an Deutlichkeit, er erfährt langsame Gestaltsveränderungen, die schliesslich einen amöboiden Charakter annehmen," and the nucleolus gradually disappears (this process lasting a half hour); "1½ Stunde später wurden in jedem Tochterkern vier kleine Nucleolen bemerkt, nach 3½ Stunden waren nur noch je zwei Nucleolen vorhanden und nach weiteren 1½ Stunden nur noch je einer. . . . Bei der Verschmelzung bilden die Nucleolen zunächst einen bisquitförmigen Körper, der sich dann später kugelig abrundet. Die Deutlichkeit der Nucleolen nimmt während des Vorganges der Verschmelzung stark ab, um später wieder zu steigen." Contrary to Strasburger and Tangl, he believes that no "Paranucleolen" wander out of the nucleus, but that where such have been observed, it has been due to the method of fixation. He notes that while the egg cells always contain nucleoli they are frequently absent in the male cells. "In alternden Zellen sind Gestaltsveränderungen, Kleinerwerden und Schwinden des Nucleolus beobachtet worden. . . . Mir scheint es nicht begründet zu sein, den Nucleolus als eine Ablagerung von Reservestoffen zu betrachten. Wesshalb sollte er nicht ein Organ der Zelle sein, wie es Flemming annimmt? . . ." Against Strasburger's views "ist zu erwidern, dass wir über das active oder passive Verhalten der Nucleolen im ruhenden Zustande oder dem der Theilung überhaupt gar nichts wissen, und das Bestehen einer Organisation für die Nucleolen ebenso gut angenommen werden kann wie für irgend einen anderen Theil der Zelle."

Meunier ('86), *Spirogyra:* the single large nucleolus has a limiting membrane, and in the fresh state contains no vacuoles, vacuoles only appearing in the dying cell, and then are probably introduced drops of water. It stains with methylen green more intensely than any other structures of the nucleus, and also stains with acid picrocarmine, alkaline carmine, and haematoxylin; "ainsi . . . on constate que les matières colorantes, reputées spécifiques de la nucléine, limitent uniquement leur action efficace et significative au corps réfringent et apparemment réticulé du nucléole." After the action of nitric acid of from 2% to 4% a reticulation is found in the nucleolus; a 10%

or 12% solution of the same acid dissolves this reticulation and only preserves the clear, non-refractive stroma; 2% or 4% hydrochloric acid solution shows the reticulation of the nucleolus to be "un boyau continu et pelotonné. . . . Le filament chromatique du nucléole ne se digére pas dans la liquer digestive [suc gastrique]. . . . Nous ne craignons pas d'affirmer que le nucléole des *Spirogyra* reproduit fidèlement, dans ses traits essentiels, la structure des noyaux les plus parfaits. Il a une membrane propre, probablement une partie protoplasmatique, quoique fort réduite; il renferme toute la nucléine du noyau, et celle-ci est exclusivement confinée dans un étui de plastine, qu'elle remplit plus ou moins complètement. . . . Quoi qu'il en soit, nucléole par position, noyau par nature, on ne peut lui refuser le nom de nucléole-noyau, dans le sens attaché à ce mot par J. B. Carnoy."

Schwarz ('87) studied the microchemistry of plant cells. He distinguishes the following substances in the nucleus: "chromatin," "pyrenin" (nucleolar substance), amphipyrenin" (substance of the cell membrane), "linin" (achromatic fibrils), and "paralinin" (nuclear sap). The pyrenin and amphipyrenin "stimmen in fast allen Reactionen überein, sie unterscheiden sich jedoch durch ihre Tingirbarkeit, indem das Pyrenin der Kernkörperchen Farbstoffe fast immer sehr leicht aufnimmt und festhält, während das Amphipyrenin nur wenig oder gar nicht tingirt wird. . . . In den weitaus meisten Fällen liegt das Maximum des Nucleolusvolumens vor der Zone, in welcher der Kern sein Maximum erreicht, und in vielen Fällen tritt gerade dann die bedeutendste Verkleinerung des Nucleolusvolumens ein, wenn der Kern sein Volumen am stärksten vergrössert. Es scheint mir demnach wahrscheinlich, dass ein Theil der Kernkörperchensubstanz direkt bei der Neubildung der übrigen Kernsubstanz verbraucht wird."

Went ('87), mitosis in various cells of plants. *Leucojum*, embryo sac: at the commencement of the prophasis there are two large nucleoli, which lie between the fibers of the chromatin network; later they become apposed to these fibers, and he notes how "die Masse des Nucleolus langsam in die des Kernfadens übergeht. . . . Im Wandbelege des Embryosackes von

*Helleborus viridis* scheinen die Nucleolen auch im Kernfaden aufgenommen zu werden"; and there is apparently the same process in *Fritillaria imperialis.* "Bei den Kernen im Wandbelege des Embryosackes von *Narcissus pseudonarcissus* findet die Aufnahme des Nucleolus ungefähr wie bei *Galanthus* statt; er wird also von allen Seiten vom Kernfaden umwunden; allmählich windet dieser sich wieder los. Oft ist dann der Nucleolus schon ganz aufgenommen, zuweilen aber werden noch Theile davon vom Kernfaden fortgeschleppt und bleiben dann wohl einmal sichtbar bis zum Anfang der Metaphase. Wenn man Präparate mit diamantfuchsin-jodgrün tingirt hat, sieht man, dass die Farbe des Kernfadens vor der Aufnahme des Nucleolus blaugrün ist, während dieser letztere roth gefärbt ist; nach der Aufnahme des Nucleolus und während der ganzen Meta- und Anaphase ist die Farbe des Fadens deutlich violett geworden, was naturgemäss verursacht ist durch die Aufnahme des Nucleolus"; also during the mitosis of similar cells in *Hyacinthus* and *Tulipa* nucleolar substance is taken up by the nuclear filament. "Ich glaube aus den hier mitgetheilten Thatsachen wohl den Schluss ziehen zu dürfen, dass in vielen Fällen wenigstens der Nucleolus beim Anfang der Kerntheilung im Kernfaden aufgenommen wird. . . . Am wahrscheinlichsten ist es wohl, dass, wo der Nucleolus vor der Theilung im Kernfaden aufgenommen wird, er sich nach der Theilung auch wieder daraus bildet."

Strasburger ('88) studied nuclear division in *Spirogyra polytaeniata.* In the resting nucleus there is usually one large nucleolus, which disappears immediately before the formation of the nuclear filaments, and by dissolving in the nuclear sap causes the latter to stain more intensely: "Als wahrscheinlich stellte ich ['84] es aber hin, dass die im Kernsaft gelöste Nucleolussubstanz den Kernfäden als Nahrung diene. . . . Auf Grund meiner neueren Erfahrungen erscheint es mir überhaupt unwahrscheinlich, dass die Nucleolarsubstanz, auch nach ihrer Auflösung im Kernsafte, den Kernfäden als Nahrung dienen sollte." In each daughter-nucleus several nucleoli arise, and these have the same number, position, and size in the two nuclei; later the several nucleoli of each daughter-nucleus unite to form

a single large nucleolus, and during this process the nuclear sap gradually loses its staining power. He shows that when the nucleolar substance is dissolved in the nuclear sap, and after the cell division, a portion of this substance plays a part in the production of the cellulose walls of the daughter-cells ; but he holds that not all of it is thus consumed, but that the nucleoli have probably some other, as yet unknown, function.

Mann ('91) introduces a new method of differential nuclear staining: when plant tissues are stained for ten minutes in saturated solution of heliocin in 50% alcohol, and then from ten to fifteen minutes in a saturated aqueous solution of aniline blue, the nucleolus is red, the rest of the nucleus and the cell blue.

Macfarlane ('92) constructs the following hypothesis, based on previous observations of his own and of Mann: "We would consider, then, that the nucleolus is the special chromatic and cell center ; that it sends out fine radiating processes — the intranuclear network — which partially fuse externally to constitute the nuclear membrane, the interspaces of the network being occupied by nucleoplasm concerned in metabolic change; that radiating continuations of the chromatic substance pass out beyond the nuclear membrane and form a network in the protoplasm, while we would suggest for future proof or disproof that they further may be continued through wall pores to form an intercellular chromatic connection. . . . We would thus view a plant as a group of connected hermaphrodite cells, . . . bound together by a fine chromatic ramification, in the center of which in each cell is the nucleolus."

Mann ('92) studied the cells of the embryo sac of *Myosurus minimus*. At the commencement of the conjugation of the two nuclei resulting in the formation of the primary endosperm nucleus, each nucleus contains "a large deeply stained nucleolus enclosed by a very faintly stained nucleolar membrane," and in each nucleus are also one or two smaller globules, which "seem to originate thus: when the nuclei about to conjugate have come in contact, one or two small nucleoli arise by the unequal division of the primary nucleolus. . . . These secondary nucleoli seem to have at first the power of division, but gradually they lose this power and their property of becoming

deeply stained, and change into globular colloid-looking masses with a central more deeply stained spot. I propose to call these bodies paranucleoli, because of their origin they may always be found in the micropylar nucleus and occasionally also in the antipodal nucleus." When these nuclei begin to conjugate, the large nucleoli of both fuse to form the single nucleolus of the primary endosperm nucleus ; at the same time a new structure makes its appearance, in close contact with the nuclear membrane of the primary endosperm nucleus : "This body . . . corresponds, I believe, to the nucleolar membrane of the antipodal nucleus" ; it is at first granular, later homogeneous. Still other, smaller spherical bodies later appear in the nucleus, which may have some connection with the paranucleoli. Finer structure of the nucleolus: in the nucleolar membrane "a number of very minute dark radially placed pores or striae can be observed, and . . . these striae are continued into very delicate cilia-like fibrils radiating out from the nucleolar membrane into the nuclear hyaloplasm. . . . The nucleolus is differentiated into an outer zone and an inner zone. The outer zone is less deeply stained, and on careful examination is found to be made up of a circle of peripheral endonucleoli, which are slightly elongated radially. The inner zone of the nucleolus is very darkly stained, and shows a number of large and irregularly disposed endonucleoli." The structure of the nucleolus may be somewhat different in other stages of its development, thus it may be composed of "(1) A thin unstained nucleolar membrane ; (2) a great number of peripheral endonucleoli ; (3) a deeply stained, apparently structureless, layer ; (4) a corona of minute, slightly elongated, endonucleoli surrounding (5) a large central endonucleolus. . . . In a resting cell, . . . the center of the nucleolus is occupied by a large endonucleolus, which sends out minute fibrils through the nucleolar substance. . . . I believe the endonucleolar fibrils probably to pass through the finer pores in the nuclear membrane" ; and Mann conjectures that "the endonucleolar filaments constitute the linin element of the chromosomes." Functions of the nucleolus: it is "concerned in the assimilation of food-material." He holds "the nuclear chromatin to be less highly elaborated

and less assimilative albuminoid material than the nucleolar chromatin. On the assumption just stated, we could explain also why we find . . . at the time of maturation portions of nucleolar matter detaching themselves from the main nucleolus to undergo a peculiar gelatinous change. The gelatinous change would correspond to a conversion of the assimilative material into achromatic elements, an explanation which would also explain the disappearance of nucleoli during the division of a cell. . . . I believe the hypothesis that the nuclear chromatin-segments and perhaps the nucleoli are organs for the conversion of assimilated material into material directly available for the achromatic elements of the cell to be not quite erroneous." In the mechanism of cell conjugation: "The endonucleolar fibers running through the body-plasm of the two sexual cells . . . are brought into contact with one another whenever the pseudopodial processes of the two cells have met. As soon as an union of fibrils has taken place, each fibril will commence to contract similarly to a muscular fibril," which results in drawing the two nuclei, afterwards also the two nucleoli, together; thus the endonucleolus is the "tropic center" of the cell.

Rosen ('92a) studied the differential staining of the nuclear elements in plants. Flowers of *Scilla:* in the nuclei of the "Bündelparenchym" are numerous large nucleoli, which differ in form and size; the one or two larger ones, "Eunucleoli," are each surrounded by a clear space, but none is present around the smaller "Pseudonucleolen." With the double stain, Altmann's acid fuchsine and methylen blue, the Eunucleoli stain red and the Pseudonucleoli blue, or *vice versa.* Similar cells of *Hyacinthus:* by the application of the double stain, aqueous solutions of fuchsine and methylen blue respectively, the Eunucleoli stain red, the Pseudonucleoli blue; but when these stains are applied in the reverse order, the nucleoli stain reversely. He considers, following Auerbach ('90), that the Eunucleolus is erythrophilic, the Pseudonucleoli kyanophilic, the latter staining as does the chromatin network. "Meine Pseudonucleolen aber sind eben offenbar weiter nichts, als besonders selbständig ausgebildete Bestandtheile des chromatischen Kerngerüstes und sind wie dieses und sein Produkt, der

Kernfaden, kyanophil"; these disappear before the mitosis, while the Eunucleoli remain until about the end of the spirem stage. Vacuoles arise only in the Eunucleoli.

Rosen in a second paper ('92b) presents further observations upon nucleoli. *Myxomycetes:* the spore nucleus contains one large nucleolus. *Fuligo septa*, plasmodium: one large, cyanophilic nucleolus, which he terms "Mittelkörperchen," since in the atypical mitosis this body lies in the middle of the pole plate, and disappears at the end of the nuclear division. *Synchrytrium:* one large nucleolus with several vacuoles; in the first mitosis the division of this nucleolus precedes that of the nucleus, but during subsequent divisions the nucleoli vanish. In *Cystopus* there is no nucleolus.

Schottländer ('92), cells of cryptogams: the nucleus consists of a blue-staining substance (network), and a red-staining (nuclear membrane, nucleoli). Egg cell of *Gymnogramme chrysophylla:* here are one or several large nucleoli, each surrounded by a vacuole; in the ripe egg the nucleoli are filled with small globules. Egg cell of *Chara:* the nucleoli contain vacuoles, which later become so large in the largest nucleoli that they become polygonally flattened against one another, and their thin walls then present the appearance of a network within the nucleolus.

Demoor ('93), mitosis of *Tradescantia:* the nucleoli gradually disappear during the prophase.

Gjurasin ('93) investigated the nuclear division of *Peziza*. In the nucleus is one large, excentric nucleolus, which stains red with Flemming's triple stain, while in it as many as six granules may occur, and these stain violet. In the mitosis these granules disappear, but otherwise the nucleolus does not change at first, but occupies its original position within the cell, though now in the cytoplasm; eventually it disappears gradually. In each daughter-nucleus a new nucleolus arises, which apparently has no genetic connection with the mother-nucleolus (now vanished). "Ich bin der Ansicht, dass . . . das Kernkörperchen nicht eine Art von Reservestoff darstellt, sondern ein specifisches Organ des Zellkernes ist."

Karsten ('93), nuclear division of *Psilotum:* in the resting nucleus are two or three nucleoli, which are homogeneous, oval

or spherical, and after haematoxylin-eosin, stain a rose color, while the chromatin is blue. At the time of the appearance of the chromosomes, "treten die Nucleolen aus den sich zusammenordnenden Plasma und lassen sich hier in Form scharf umschriebener, homogener, roth gefärbter Kügelchen nachweisen." Usually two nucleoli wander out, at least never more than two were found outside of the nucleus. These two come to lie at opposite poles of the nucleus, occupying the positions of centrosomes; and when the longitudinal splitting of the chromosomes takes place, each of the nucleoli also divides into two. Karsten believes these nucleoli are identical with the centrosomes of Guignard; but he does not explain what becomes of the third nucleolus during the division.

Lauterborn ('93), quoted by Karsten ('93), diatoms: there is a centrosome lying in a concavity of the nucleus; he noticed, further, "beim Beginn der Theilung aber zwischen Kern und Centrosom noch ein anderes Gebilde —, welches im späteren Verlauf der Karyokinese eine sehr bedeutsame Rolle spielt, nämlich die Anlage der Centralspindel"; this body must be derived either from the nucleus or the centrosome (I mention it here since it may in the future be found to have some connection with a nucleolus).

Moll ('93) studied karyokinesis in *Spirogyra*. There are one or two nucleoli, which stain more intensely with gentian violet than any other portion of the nucleus. They may be vacuolar in structure, or contain a skein of chromatin; they appear homogeneous only when too deeply stained. The skein structure (the skein itself staining as chromatin) is found in resting nuclei, as well as in the prophases of mitosis, and at the same time vacuoles may be present. He assumes that the thread in the nucleolus contains all the chromatin of the resting nucleus, and "that by the nucleolus the chromatin substance for the segments [chromosomes] is furnished"; this chromatin leaves the nucleolus in mitosis, and "it seems as if the chromatic substance were squeezed from the nucleolus by an aperture." After the chromatin skein has left the nucleolus, the latter disappears.

(Strasburger's paper, '93, was reviewed under the head of zoölogical literature.)

Wager ('93), nuclear division in *Hymenomycetes agaricus:* each nucleus of a basidium contains one large nucleolus, besides the nuclear network. The two nuclei of the basidium fuse together and form one nucleus, in which afterwards the two nucleoli later fuse to form one nucleolus. This latter is often vesicular in structure. In the mitosis it lies close to the nuclear membrane, it gradually loses its staining intensity, decreases in size, and finally disappears; at the same time the cytoplasm in its neighborhood stains more deeply. But sometimes it persists until the diaster stage. "From the fact that the chromosomes begin to stain red at the time of the disappearance of the nucleoli, it would further appear that the former can take up nucleolar substance from the nuclear sap, and as fast as the nucleoli disappear the chromatic elements become more deeply stained red." In *A. stercorarius*, in the daughter-nucleus, "the chromatin mass appears to be transformed at once into the nucleolus," and only later a chromatin network appears. "I would suggest that the nuclear threads take up the dissolved nucleolar substance at some period during the division, and carry it over into the daughter-nuclei, to be given up again later as the nucleoli of the latter. . . . But a certain quantity of the dissolved nucleolar substance probably escapes into the cytoplasm when the nuclear membrane disappears, and this would be taken up at a later stage into the daughter-nuclei, as is shown by the increase in size of the nucleoli, and by the decrease in the capacity of the protoplasm for taking up stains."

Zacharias ('93) finds in plants that the nucleolus and cytoplasm are erythrophilic, the nuclein (chromatin) network is cyanophilic.

Belajeff ('94), "Pollenmutterzellen" of *Larix:* after the disappearance of the nuclear membrane in mitosis the nucleolus becomes gradually smaller and then disappears; several nucleoli reappear within each of the daughter-nuclei. "Es ist zu bemerken, dass nach der Auflösung der Nucleolen der Mutterzelle im Zellplasma eine gewisse Anzahl grober Körnchen erscheint, welche mit Safranin färbbar sind. Mit dem Beginn der Nucleolenbildung in den Töchterzellen verschwinden die Körnchen vollkommen. . . . Ich erklärte mir die Ergebnisse

meiner Beobachtungen derart, als lösten sich die Nucleolen, nach vorausgegangener Auflösung der Kernmembran, unter der Einwirkung der in die Kernhöhle aus dem Zellplasma gedrungener Substanzen, gänzlich auf, um später durch den Einfluss des Kernsaftes, der die ganze Zelle durchdrungen, wieder hergestellt zu werden, indem der Kernsaft die Nucleolensubstanz im Zellplasma so zu sagen gerinnen macht. Nach der Bildung der Töchterkerne, welche ihren Kernsaft aus dem Zellplasma absorbiren, werden die Körnchen abermals vom Zellplasma aufgelöst, um zum zweitenmal im Inneren der jungen Kerne (Töchterkerne) in der Gestalt von Nucleolen zu erscheinen." In *Fritillaria* and *Lilium* also the nucleolus is dissolved after the disappearance of the nuclear membrane.

Humphrey ('94) studied the "Pollen-" and "Sporenmutterzellen" of *Convalleria*, *Ceratozamia*, *Osmunda*, and *Psilotum*, and cells from the apex of the root of *Vicia* and *Hyacinthus*. The nucleolar substance is usually not to be found in the cytoplasm during mitosis. The nucleoli are "keine individuellen Bestandtheile, sondern unbestimmte Massen von Nucleolarsubstanz, und ihr Vorkommen im Cytoplasma hat keine weitere Bedeutung als zu zeigen, dass eine Communication zwischen Kernhöhle und Cytoplasma bisweilen, wenn auch nicht immer, sich herstellen kann und dass entweder die Nucleolen in einigen Fällen aus der Kernhöhle, bevor sie von den karyokinetischen Kräften angegriffen werden, austreten können, oder dass die Menge der Nucleolarsubstanz in einem Kerne grösser sein kann, als diese Kräfte zu lösen oder zu verbreiten vermögen. . . . Die 'Vacuolen' der Nucleolen scheinen mir das natürliche Resultat der nachherigen Trennung der flüssigeren von den festeren Theilen der Nucleolarsubstanz zu sein. . . . Wenn also Zimmermann ['93] den Satz aufstellt 'Omnis nucleolus e nucleolo,' so kommt er zu einer Verallgemeinerung, die nicht zulässig und derjenigen 'Omnis nucleus e nucleo' nicht gleichwerthig ist." In every nucleus of the "Pollensäcke" of *Ceratozamia* there is a large, peripherally placed paranucleolus (Strasburger): "In extremen Fällen kann die Anhäufung von Substanz eine so grosse sein, dass die Kernmembran hier bedeutend hinausgestossen wird. . . . Auf der Fuchsin-

Jodgrün tingirten Schnitten werden die Paranucleolen weder reinroth wie die Nucleolen, noch blaugrün wie die chromatische Substanz gefärbt, vielmehr nehmen sie eine Zwischennuance, welche mehr der des Chromatins als der der Nucleolen ähnelt, an"; he believes these paranucleoli to be artefacts. In contradiction to Karsten ('93) he found no body in *Psilotum* comparable to a Nucleo-Centrosoma.

Zacharias ('94) concludes, from numerous observations on cells of plants that as the size of the nucleus increases (or decreases) with the size of the cell, so also that of the nucleolus increases (or decreases) with the size of the nucleus.

In Rosen's ('95) contribution a large number of new facts are recorded, which may be briefly mentioned. The kyanophilic nucleoli of Auerbach "sind eben keine Nucleolen und bedürfen als wenig constante Theile des Chromatingerüstes überhaupt keines besonderen Namens." *Hyacinthus:* in meristem nuclei all the nucleoli except the smallest lie in special clear spaces, and though fibrils are rarely found in connection with them, "gleichwohl muss das Kernkörperchen in seiner scheinbar schwebenden Lage wohlbefestigt sein, da es . . . stets seine Lage im Centrum seines Hofes bewahrt." The large nucleoli of the "Gefässzellen" become vacuolar as they increase in size. In mitosis of root cells the nucleoli become gradually dissolved within the nucleus in some species, in others they are extruded into the cytoplasm; in the latter cases "erfolgte die Zerklüftung und Auflösung des Nucleolus viel langsamer, sodass bei dem Schwinden der Kernmembran noch bedeutende Nucleolarreste vorhanden waren." The nucleoli reappear in the dispirem stage before the daughter-nuclei have produced membranes, and the new nucleoli stain from the commencement intensely; from which the general conclusions are drawn: in the prophase the diminishing nucleolar substance penetrates, perhaps as a micellar solution, into the cytoplasm, and this process may cease before the nuclear membrane has disappeared. In some cases larger particles of nucleolar substance may penetrate into the cytoplasm, but only after the nuclear membrane has disappeared, and these particles become subsequently dissolved in the cytoplasm; in either case "das lösende Agens muss wohl

der Kernsaft sein, vielleicht unter Mitwirkung eines nur während der Prophasen gebildeten Enzyms. Während der Anaphasen wandert die Nucleolarlösung als solche in den Raum der Tochterkerne ein, und hier wird die Nucleolarmasse wieder fest. Bei der Hyacinthe — und anderen Objekten — erfolgt die Rekonstituirung der Nucleolen auch ausserhalb der Dispiremfigur. Die derart im Cytoplasma entstandenen Nucleolen wandern, wie ich glauben möchte, in die Tochterkerne ein, ehe sich diese mit einer Kernmembran umhüllen; wenn letzteres geschehen ist, so findet man anscheinend niemals mehr Nucleolen im Cytoplasma, die, wenn überhaupt, auch wohl nur nach nochmaliger Auflösung in den Kernraum gelangen könnten. Nicht ganz unmöglich scheint es mir, dass die Nucleolen, die man an fixirten Präparaten . . . im Cytoplasma auffindet, doch durch die coagulirende Wirkung des Fixirungsmittels entstanden sind. Ich glaube aber, dass dies von keiner grossen Bedeutung ist, denn an den Stellen, wo wir extranucleäre Nucleolen vorfinden, muss dann die Masse der Kernkörperchen als Lösung angesammelt gewesen sein." Also in the mitosis of root cells of *Aspidistra*, are nucleolar fragments seen in the achromatic spindle. Root cells of *Phaseolus:* in the resting stage there is a single nucleolus; in the mitotic prophase it becomes first lobular, then lengthened in the direction of the spindle, while at the same time it is undergoing a slow dissolution; "wenn die Spindel gebildet und die Kernwandung verschwunden ist, sieht man fast stets inmitten der zur Kernplatte angeordneten Chromosomen einen mehr oder minder ansehnlichen Nucleolarrest, welcher in derselben Richtung wie die Chromosomen und die Spindelfaden gestreckt ist. Dieser Nucleolarrest wird nun in der Mitte eingeschnürt, sodass er Hantelform erhält; die beiden Hälften reissen schliesslich von einander und gelangen an die Spindelpole. In anderen Kernen wird der Nucleolarrest einseitig aus der Kernplatte herausgedrängt oder auch doppelt getheilt; endlich findet sich meist an einem oder an beiden Spindelpolen ein Restchen des Nucleolus; seltener liegt ein solches neben der Spindel. Die Auflösung ist nun meist bald beendigt"; and only exceptionally is there a minute nucleolar remnant in the cytoplasm at the end of

mitosis. "Unzweifelhaft sind auch bei Phaseolus multiflorus die Nucleolen der Tochterkerne Neubildungen. Wenn auch die Nucleolarsubstanz möglicherweise bei der Karyolyse erhalten bleibt und sich in den Tochterkernen nur wieder auf Neue sammelt, so besteht doch keine von Generation zu Generation sich fort spinnende Continuität in den Nucleolen als solchen und von einem 'omnis nucleolus e nucleolo' [Zimmermann] kann keine Rede sein." Root cells of *Vicia faba:* the nucleolar mass diminishes as the cell degenerates; "dieselbe stellt das erste Zeichen der Kerndegeneration . . . dar und ist, wie sonst, mit einer Zertheilung des Nucleolus verbunden," while a large nucleolus surrounded by a clear space is an embryonic condition. In the mitosis of these cells no nucleolar fragments pass into the cytoplasm, and in each daughter-nucleus two nucleoli arise which subsequently fuse into one. In opposition to Lavdowsky ('94), he contends that the centrosomes have no genetic connection with nucleoli, and that the nucleolar substance does not serve as nourishment for the chromosomes; "nichtsdestoweniger wäre es voreilig zu behaupten, dass von der Substanz der Nucleolen nichts in die Fadensegmente gelangen könne. . . . Die Violettfärbung der Segmente in den späteren Phasen der Karyokinese . . . könnte auf eine Einlagerung erythrophiler Nucleolarsubstanz in den kyanophilen Kernfäden schliessen lassen." In buds of *Psilotum triquetrum* the nucleoli are excentric, while in most plants they have a central position. In the mitosis nucleolar fragments are extruded into the cytoplasm (in agreement with Zimmermann, in opposition to Karsten and Humphrey), and none of the extruded masses can be regarded as centrosomes (against the view of Karsten). Three nucleoli usually arise in each daughter-nucleus: "Sie entstehen nahe der Peripherie des jungen Kerns, oft in Contakt mit dem Cytoplasma, bevor die Tochterkerne sich mit einer Membran umschliessen und verschmelzen später nicht miteinander." In the mitosis of sporangia the nucleoli are usually "aus den karyokinetischen Figuren ausgestossen"; and the "Secretkörperchen" of Strasburger is a true extruded nucleolus.

Strasburger ('95, cited by Lauterborn, *Zool. Centralbl.*, 1896)

concludes that the nucleolar substance, dissolved in the nuclear sap, may be used in the production of the spindle fibers.

Koernicke ('96), study of mitosis on *Triticum:* in the development of the embryo sac when the two pole nuclei fuse together, the two nucleoli also join to form one. In the mitosis of the pollen the nucleolus always disappears before the formation of the spindle, but it could not be determined whether it takes any part in the formation of the latter.

Lauterborn ('96), nuclei of diatoms: there are several nucleoli present; in the spirem stage of division they commence to gradually disappear; "es scheint mir ziemlich sicher, dass ihre Substanz mit derjenigen der Chromatinkörnchen und des Liningerüstes zur Bildung der Knauelfaden verbraucht wird." It is important to note that the central spindle arises outside of the nucleus, before the nucleoli begin to disappear, so that there can be no genetic connection between the two.

Poirault and Raciborski ('96), binucleated ("conjugate") *Uredineae* during the production of the ascidiospore generation: in the mitosis the nucleolus becomes extruded into the cytoplasm, almost always in the equatorial plane. "Bei manchen Arten bleiben sie sehr lange erhalten so z. B. bei *Peridermium Pini acicola*, wo neben den längst ruhenden, mit neuen Nukleolen versehenen Kernen noch in den Plasma, die alten Kernkörperchen der Elternkerne herumirren. Mit den Centrosomen haben somit diese extranukleolären, vakuolirten Nukleolen nichts zu thun."

Zimmermann ('96), a general critical summary upon the vegetable nucleolus, with consideration of a part of the previous literature. Nucleoli are almost always present in the cells of the higher plants, and are of wide occurrence also in the lower forms; double staining serves to differentiate them from the chromatin. There are usually from one to three to a nucleus, but in the embryo sac of *Lilium martagnon* there are from twenty to thirty. In *Chara* the older nuclei show the nucleolar substance in the form of very numerous, irregular fragments. The distinction of "Hauptnucleolus" and "Nebennucleolus" is not tenable, since the latter may be possibly chromatin globules. "Mit dem Chromatingerüst scheinen die Nukleolen

innerhalb der ruhenden Kerne in keinem Falle in direkter Verbindung zu stehen." The space frequently observed around the nucleolus is probably not an artefact. Its substance is probably homogeneous; "als die alleinigen mit Sicherheit nachgewiesenen Einschlüsse derselben können Vakuolen angeführt werden. . . . Diese Vakuolen sind dem gewöhnlichen Einschluss in Kanadabalsam häufig ganz oder teilweise mit Luft erfüllt oder stellen luftleere Räume dar. Sie erscheinen dann bei höherer Einstellung schwarz, bei niederer etwas rötlich, und es dürften wohl die namentlich in der die Kerne beiläufig behandelnden Litteratur vorliegenden Angaben über stark lichtbrechende Einschlüsse der Nukleolen zum Teil auf derartige Bilder zurückzuführen sein" (*e.g.*, the "endonucleoli" described by Mann). During mitosis nucleolar bodies are often found in the cytoplasm, and such are probably extruded nucleolar fragments; "immerhin muss aber die allgemeine Gültigkeit des früher von mir als möglich hingestellten Satzes *omnis nucleolus e nucleolo* nach den neueren Untersuchungen als nicht sehr wahrscheinlich angesehen werden." In the Pollenmutterzellen of *Lilium martagnon* the nucleoli "zerfallen . . . in sehr zahlreiche kleine Kugeln, die . . . im Asterstadium ungefähr gleichmässig über den gesammten Zellinhalt zerstreut sind." He made similar observations also on *Hyacinthus candicans*, *Fritillaria imperialis*, young sporangia of *Equisetum* and *Psilotum*, cells of the root apex of *Vicia*, and stem apex of *Phaseolus* and *Psilotum*. There is also an extrusion of nucleolar substance in *Chara*, but it is doubtful whether this process occurs in other low forms. This extruded substance may in some cases, but perhaps not as a rule, return into the daughter-nuclei. That in mitosis the nucleolar substance may be incorporated into the chromosomes, "sei noch erwähnt, dass ich neuerdings an den Kernteilungsfiguren des Embryosack-Wandbelags von *Lilium martagnon* nach der Fixierung mit Chromsäure und Platinchlorid und Färbung mit Fuchsin und Jodgrün in den Endstadien des Spirems beobachten konnte, dass einzelne rote Kugeln, die ausserdem auch in grosser Zahl in der Umgebung der betreffenden Kerne zu beobachten waren, den violettgefärbten Chromosomen teils

seitlich ansassen, teils auch ganz von denselben aufgenommen waren, so dass sie . . . kleine Auftreibungen an denselben bildeten." It is doubtful whether the nucleoli have any genetic connection with either the centrosome or the nuclear membrane. In the synapsis (Moore, '95) of the nucleus the nucleolus becomes flattened against the nuclear membrane in most *Angiospermia*, having thus on section a sickle shape ("Sichelstadium") ; and the coincidence of this form of the nucleolus with the synaptic stage "macht es jedenfalls sehr wahrscheinlich, dass die im Sichelstadium eintretenden Metamorphosen den Nukleolus eine gewisse Bedeutung besitzen."

Dębski ('97), *Chara:* the space surrounding the large nucleolus is caused by shrinkage of the latter, due to the fixing fluids, and is not present in life. In the nucleolus are numerous vacuoles which may become confluent. Within the cytoplasm occur extranuclear nucleoli, which stain like the others. In the mitotic prophase the nucleolus usually divides into two, and the latter either gradually diminish in size and finally disappear or else they persist for a while after the disappearance of the nuclear membrane. Then the extranuclear nucleoli collect at the poles of the spindle and "bewegen sich während der Metakinese von beiden Seiten her gegen den Ort der späteren Zellplattenbildung und verschmelzen dabei nicht selten während des Diasterstadiums miteinander zu unregelmässigen Kugeln, Klumpen und Fäden . . . die nucleolenartigen Körper sind später, nach der Bildung der Zellplatte und der Membran, nicht mehr dort zu sehen ; es finden sich alsdann nur noch wenige durch das ganze Plasma der Zelle zerstreut, oder sie fehlen, besonders in den älteren Zellen gänzlich. Einige, wahrscheinlich solche, welche während des Diasters nicht in die Zellplattenebene gerückt sind, finden sich während des Dispirems in der Nähe der Tochterkerne ein ; später sind sie zwischen den Fäden des Kerngerüstes zu sehen ; in späteren Stadien findet man an ihrer Stelle einige kleine Nucleolen, deren Zahl immer mehr beschränkt wird, so dass sich schliesslich gewöhnlich in jedem Kern ein einziger grosser Nucleolus befindet."

Fairchild ('97), *Basidiobolus:* "Das Verschwinden des

Kernkörperchens . . . spricht entschieden für Strasburgers Annahme, dass es zur Bildung der Spindelfasern benutzt werde."

Harper ('97), ascus of *Erysiphe:* the nucleolus and the centrosphere stain in the same way, and "die achromatischen Fasern, aus welchen diese intranucleären Strahlenkegel gebildet werden, entstehen wahrscheinlich grösstentheils auf Kosten der Kernkörperchensubstanz, die zu dieser Zeit regelmässig verschwindet."

Huie ('97), cells of *Drosera:* the nucleoli ("nucleolar chromosomes") are spherical and usually central; "endonucleoli" are enclosed spaces, not granules. During the process of food assimilation by the nucleus the nucleolus becomes smaller, and its vacuoles less apparent.

Lidforss ('97) gives a thorough review of the "Sichelstadium" (Strasburger's "Sekretkörperchen") of the nucleolus in plant cells, as also the results of observations of his own on the embryo sac. *Tulipa:* at first there are several small nucleoli within the nuclear cavity, which later by their fusion produce a large one which becomes flattened against the nuclear membrane (the process is essentially the same in *Fritillaria, Anthericum*, and *Lilium*). *Gagea:* the nucleolar changes are as in the preceding forms, except that when the nucleolus reaches the periphery it remains spherical; this is also the case in *Ornithogalum*. *Oenothera:* in the youngest cells there is one central nucleolus; subsequently this flattens against the nuclear membrane, but finally wanders back to the center and becomes spherical. He concludes that in the angiosperms the sickle stage of the nucleolus is a normal phenomenon, as is also its excentric position. In male and female germ cells these metamorphoses occur at corresponding stages, namely, when the reduction of the chromatin takes place; "indessen bleiben vorläufig alle Speculationen über die Bedeutung des Sichelstadiums von problematischen Werth. . . ."

Mottier ('97), cells of *Podophyllum* and *Lilium:* in mitosis, at the time of disappearance of the nuclear membrane, the nucleolus breaks into fragments of various size. "Bei der Anlage der vielpoligen Spindel nun treten im Cytoplasma

kleinere, dem Nucleolus ähnlich tingirte Körper auf. . . . Es unterliegt keinem Zweifel, dass dieses die zerfallene Kernkörperchensubstanz darstellt . . . nachdem der Tochterkern mit einer Wandung versehen wurde und in ihm Kernkörperchen zum Vorschein kamen, sind oft noch extranucleäre Nucleolen in dem Cytoplasma zu sehen. Dasselbe gilt für die zweite Theilung. Ob die in dem Tochterkern zum Vorschein kommenden Kernkörperchen aus den im Cytoplasma liegenden Körperchen entstehen, lässt sich nicht feststellen. Hingegen wäre hier hervorzuheben, dass die im Kern wieder entstehenden Kernkörperchen stets in Contakt mit den Kernfäden sich befinden . . . meine Ansicht geht aber dahin, dass in den Kernkörperchen ein Kraftvorrath gegeben ist, welcher der Zelle nach Bedarf zur Verfügung steht."

Pennington ('97), cells of *Spirogyra* treated with .1478% palladious chloride : "The nucleolus showed a dark bounding layer of double contour. . . . The dark layer is undoubtedly a true membrane dividing the nucleolus from the nucleus."

Strasburger ('97) reiterates his view ('95) that in plant mitoses the achromatic spindle is formed from nucleolar substance, and that also the "Zellplatte" and "Centralspindelkörperchen" of animal cells must be of nucleolar origin.

Swingle ('97), algae (*Sphaelariaceae*) : the vacuolization of the nucleoli occurs simultaneously with the separation of the two centrosomes, and probably at the same time that the differentiation of the chromosomes occurs. Though "die schnelle und vollständige Auflösung der übrigen Substanz des stark vacuolisirten Kernkörperchens findet statt, wenn die Spindelfasern an den Polen einzutreten beginnen," there yet seems to be no direct proof that these fibers have their origin in nucleolar substance. "Könnte er [Nucleolus] nicht eher einen speciellen Vorrath organischer Nahrung zur Erhaltung des Kinoplasmas während der Karyokinese vorstellen?"

### *C.* Synonyms of the Term Nucleolus.

Since there are quite a large number of synonyms of the nucleolus, they may for convenience' sake be classified together

at this place. Certain of the following terms, however, apply not to the true nucleoli but to the *Caryosomata.*

*German writers.* — Nucleolus (Valentin) Keimfleck, Keimkern, macula germinativa (Wagner); Kernkörper (chen) (Schwann, Valentin); Keimkörper (chen); Wagner'scher Fleck; Binnenkörper (Rhumbler); Hauptnucleolus, Nebennucleolus (Flemming); Metanucleolus (Häcker); Plasmosoma (Ogata); Formationsnucleolus (Marshall); Kernfleck, Nucleolide, Morulit (Frenzel); Nucleolo-Centrosoma (Keuten); Mittelkörperchen, Eunucleolus (Rosen); Nucleolkörperchen (Lönnberg); Stammnucleolus, Nebenkügelchen (Auerbach); Hauptkeimfleck, Nebenkeimfleck (Leydig); Chromatin-Nucleolus, Paranucleolus (R. Hertwig).

*English and American writers.* — Wagnerian vesicle, entoblast (Agassiz); pronucleolus (Mark); nucleole, germinal spot, germinal dot, principal nucleolus, accessory nucleolus, protomacrosome (Greenwood).

*French writers.* — Nucléole, tache germinative; pseudonucléole (Van Beneden); tache de Wagner, nucléole plasmatique, n. mixte, n. nucléinien, nucléole-noyau (Carnoy); nucléole adventif (Roule); corps nucléolaire, nucléolite (A. Schneider); nucléole primitif et secondaire (Carnoy and Lebrun); corpuscule germinatif (Van Beneden).

*Italian writers.* — Macchia germinativa, macchia germinativa principale, m. g. laterale, m. g. accessoria.

*Synonyms of the nucleolinus.* — Nucleololus, Nucleollolus (Frenzel); Schrön'scher Korn, Valentinian vesicle, entosthoblast (Agassiz); Centrosoma (Lavdowsky); nucleolo-nucleus, endonucleolus (Macfarlane); Nucleolinus, Keimpunkt, punctum germinativum (Haeckel).

## III. OBSERVATIONS.

### *A.* METHODS OF STUDY.

The following observations have been made upon material collected, fixed, stained, and sectioned by myself, with the exception of the preparations of the ova of *Rodalia,* which were kindly loaned to me by Dr. E. G. Conklin. In no case were observations made upon the living tissue; however, but

little could be gained from a study of the living cells, in regard to the minute structures with which we are chiefly engaged. With only few exceptions (*Rodalia* and the two gregarines examined) no cells were studied which had not been preserved with at least three fixing reagents, and in some cases at least half a dozen different fixatives were used. The preserving reagents employed were the following: saturated solutions of corrosive sublimate in distilled water (this being the only fluid used hot), sat. sol. of the same in 50% or 35% alcohol, Flemming's stronger fluid (chromo-aceto-osmic acid), Hermann's fluid (platinum chloride, acetic acid, osmic acid), sat. sol. of picric acid in 50% alcohol, Perenyi's fluid (chromo-nitric acid), 2% aqueous sol. of chromic acid, absolute alcohol, picro-nitro-osmic acid. Those reagents which gave the best general results were the fluids of Flemming and Hermann, and the alcoholic solution of corrosive sublimate ; though the particular reagent demanded depends both upon the object of study, as well as upon the method of staining which is to follow. It is hardly necessary to state that a structure found after the use of a given fluid, but not apparent on material treated in a different manner, was either regarded as an artefact, or doubts were expressed as to its naturalness ; that is, only when a structure was found to present itself to the eye in more or less the same manner, after various methods of preservation had been employed, have I regarded it as a natural appearance and not as a result of the fixatives used. Thin serial sections were cut of objects imbedded in paraffin, in the usual way. All staining done was upon the sections on the slide, and the stains employed were as follows : Ehrlich's or Delafield's haematoxylin followed by eosin (sat. sol. in distilled water), nigrosine (a sat. sol. in water diluted by six vols. water), sat. sol. of acid fuchsine in 50% alcohol, the triple stain of Ehrlich-Biondi-Heidenhain (as prepared by Grübler, Leipzig), Flemming's triple stain (safranin, gentian violet, and orange G.), Lyons blue (sat. sol. in 50% alcohol), gentian violet (sat. aqueous sol.), methylen blue (sat. aq. sol.), brasilin (sat. sols. in water and in 35% alcohol), Mayer's acid carmine, cochineal (sat. sol. in 70% alcohol) ; while Grenacher's borax carmine and alum carmine, Heidenhain's iron haematoxy-

lin, indigo-borax carmine (Norris and Shakespere), and certain others were tried, but proved unsatisfactory. With the exception of the three triple stains mentioned, the others were used in various combinations as double stains ; worthy of recommendation are (with especial regard to the differentiation of the nucleolus) Delafield's, or better, Ehrlich's haematoxylin followed by eosin ; acid carmine followed by nigrosine ; methylen blue followed by brasilin. Other combinations were also used, but it is not necessary to mention these here, nor to speak of the duration of the staining baths, since in the explanation of the figures these data are given for each case separately.

For the study of the finer structural details, the $\frac{1}{12}$th homogeneous immersion lens of Zeiss was used, in combination with oculars 2 and 4. I would emphasize the fact that the drawings from the preparations were made gradually, as I proceeded in the study of each particular cell, and were not postponed until the end of the particular investigation, so that almost all were made before I had arrived at any views upon the nature of the nucleolus ; and I have pursued this method in order to eliminate from the figures as much as possible of the subjective element. In other words, I have made as close copies as possible of the preparations, drawing every cell or structure presenting some appearance with which I had not as yet become acquainted, or rather the significance of which I had not learned, and then from the figures so made I have endeavored to learn the nature of the phenomena there presented, at the same time recurring to the preparations themselves. This method of study is the one employed by many investigators, though it can scarcely be termed the one most in vogue. The colors of the original figures have on the whole been most excellently reproduced by the lithographs of Werner and Winter.

## *B.* Protozoa.

### 1. *Gregarine from Lineus gesserensis* (O. F. Müll).

(Plate 21, Figs. 1–19.)

(*Description of the animal.* — The largest individuals are just visible to the naked eye, and are of a whitish color. No synzigia were observed among the thirty individuals exam-

ined. Form : elongate, slightly larger at one end than the other, the thinner end sometimes flattened, slightly curved or sickle-shaped ; the greatest diameter is found in the region of the nucleus, which is situated nearer to the larger than to the smaller end ; both ends of the animal are rounded. In one individual (Fig. 2) the surface of the body was slightly furrowed in a spiral direction. Nucleus large, with a very thick membrane, and seldom oval, usually irregular in outline. In a single case (Fig. 1) two nuclei were present in one gregarine (the youngest individual seen), the two nuclei were of unequal size, though each contained a single nucleolus. Kölliker ('49) has described a gregarine with two nuclei ; I am unacquainted with any other cases. Sporocysts were not observed ; but in one case the cytoplasm was quite densely filled with minute spherical and oval bodies, which stained lightly with eosin, and in each occurred a small granule (this staining with haematoxylin) ; in the same individual a normal nucleus was also present (Fig. 4). These small bodies cannot be other than spores, even though they occur in the endoplasm of a gregarine in which a nucleus occurred at the same time ; this observation stands in no accord with what has thus far been described of the sporulation among gregarines, and I am thoroughly at a loss to explain the phenomenon. These gregarines occurred only in the posterior intestine of *Lineus*, but were not present in all the individuals of this nemertean sectioned. The absence of synzigia, the transverse furrows of the body, and the oval-shaped spores would relegate this form to the neighborhood of the genus *Gonospora* of Schneider.)

In the smallest nuclei found (the size of the nucleus stands in some degree in proportion to that of the animal) only one nucleolus was present (Figs. 3 and 5) ; in all the larger nuclei their number varied from two to four, though since four nucleoli were found in only two cases, two or three nucleoli may be regarded as the usual number in the larger individuals. As an inspection of Figs. 3–19 shows, the comparative size of the nucleoli within the same nucleus is very variable, and the nucleoli of one nucleus are always of unequal size. When only two nucleoli occur, one is about one-half or three-quarters the

size of the other; but when three nucleoli are present, either (1) one is particularly large, and the other two small; or (2) two are large, and the third is much smaller than either; or (3) all three are large, the smallest being about one-half the size of the largest. In the two cases of nuclei with four nucleoli apiece, in the one there were two larger and two smaller nucleoli, in the other one large and three small ones.

The nucleoli vary from a spherical to an oval shape. In the smallest usually no vacuoles (*n. Vac.*) are to be seen, but such vacuoles are always to be found in the larger nucleoli. In the largest there is usually a large excentric vacuole, while small ones may or may not be present in other portions of the nucleolus. In nucleoli of medium size it is most usual to find a number of small vacuoles. These vacuoles have already been noticed in numerous other gregarines, but I would call especial attention to a remarkable polarity of the nucleolus with regard to their position. In all those nucleoli in which vacuoles occurred, with the exception of not more than five or six, the single large vacuole, or the group of smaller ones, was situated at that pole of the nucleolus nearest the nuclear membrane (Figs. 7–9, 16, 17–19). There are almost no exceptions to this phenomenon in the smaller nucleoli, those, namely, in which only a single small vacuole or a few small ones are present. Accordingly, it would seem to be the rule that the vacuoles first appear in that portion of the nucleolus which approaches nearest to the nuclear membrane. The number and size of these vacuoles increase with the size of the nucleolus; or, as is more usually the case, as the nucleolus increases in size they gradually fuse together to form a single large vacuole, which may occupy the greater part of the nucleolus (Fig. 15). Thus the vacuoles first arise at one point in the nucleolus, so that here one can speak of a polarity of the nucleolus; but as the vacuoles increase in number and commence to fuse together the fluid substance of them begins to diffuse more widely throughout the nucleolus, so that evidences of this primitive polarity gradually become obliterated.

The ground substance of the nucleoli is very finely granular, and stains deeply red with eosin, and brownish red with the

Ehrlich-Biondi stain. The vacuoles are filled with a structureless fluid, which stains but lightly. But in four nuclei, the sections of which were stained in aqueous solution of methylen blue followed by brasilin, a differential stain of the ground substance was acquired: that pole of the nucleolus which contained vacuoles was stained a bluish green (methylen blue), the opposite pole, where no vacuoles could be seen, being of a light pinkish color (brasilin), the vacuoles themselves appearing as clear unstained spaces (Figs. 17–19). In one nucleus, in which two minute nucleoli were present, the one without, the other with, a single small vacuole, both nucleoli stained a bluish green throughout (Fig. 18). Further, in an unstained nucleus fixed with Flemming's fluid a somewhat similar differentiation was visible in the two larger nucleoli (neither of which contained vacuoles), the pole of each nucleolus nearest the nuclear membrane being of a deeper color than the opposite pole (Fig. 11). This differentiation produced by staining would show that the ground substance of the smallest nucleoli is homogeneous, but that in the larger ones a chemical change takes place in it, whereby that portion of the substance opposite the pole where the vacuoles first appear differentiates itself chemically from that portion of the ground substance lying at the latter pole. Unfortunately I had too little material to carry further the study of this differentiation.

In the nucleus is a faintly staining nuclear sap, in which irregular granules of various size are massed together especially near the center of the nucleus; they do not come into contact with the nucleoli, usually leaving a clear space around each of the nucleoli (Figs. 7, 8, 11, 14, 17–19). These do not stain with haematoxylin or with methylen green, but stain red with eosin and brownish red with the Ehrlich-Biondi mixture, in their staining differing little from the substance of the nucleoli. With the methylen-blue-brasilin stain mentioned above they stain pink, a little more deeply than does the inner pole of each of the larger nucleoli (Figs. 17–19). Whether they represent physiologically chromatin, or whether they are masses of (perhaps nutritive) substance taken into the nucleus from the cytoplasm, which might be chemically and genetically

akin to part of the substance of the nucleoli, I am unable to decide. I am also unable to determine from the preparations at hand whether the nucleoli themselves are partially composed of chromatin ; but the usual diagnostic stains for chromatin do not show the presence of this substance within the nucleus.[1]

To revert again to the polarity of the nucleoli. The fact that the vacuoles first arise in that portion of the nucleolus nearest the nuclear membrane would seem to prove that the substance of these vacuoles is extranuclear in origin, or else is secreted in the peripheral portion of the nucleus. But since it would be obscure how the peripheral portion of the nucleus should secrete a substance, and the central portion should not, I incline to the former explanation, namely, that the substance of the vacuoles is first produced in the cytoplasm, and then this substance penetrating through the nuclear membrane, it, or a part of it, arrives at that pole of the nucleolus nearest the nuclear membrane, and then is taken into the nucleolus at this pole. The size of the vacuoles stands in a more or less direct ratio to the size of the nucleolus itself ; at the same time the ground substance of the nucleolus also increases in amount, though apparently not as rapidly as the amount of the vacuolar fluid.

### 2. *Gregarine from Carinella annulata.*

(Plate 21, Figs. 20-35.)

(*Description of the animal.*—Monocystid gregarines occurring in the body cavity of this nemertean. No synzigia observed. Form : elongate, though not attenuate, the end in which the nucleus lies being broader and terminally more obtuse than the opposite end (Figs. 20 and 21). The longitudinal axis is never perfectly straight, and the cuticula shows no transverse furrows. The single nucleus is usually spherical or oval, rarely lobular in outline. In the entosarc of many individuals occur numerous minute, refractive granules. Neither cysts nor spores having been observed, I was unable to determine the genus of

[1] However, the chromatin here might exist in the state in which it is found in the growth period of ovocytes, namely, commingled with plastin.

this form. Only two individuals of *Carinella* were examined (both from Bergen, Norway); in the one all the gregarines were large, in the other of a smaller size.)

The nucleoli are nearly always more numerous than in the preceding species of gregarine, the number varying from four to about twenty-six, in those stages found (Figs. 22–35). In the larger nuclei they are usually more numerous than in the smaller ones, but exceptions to this rule are quite frequent. In the same nucleus some are nearly or quite spherical, others very irregularly lobular in outline. Their size within a given nucleus is also very variable, though as a rule they are unequal in their dimensions. In the larger nuclei the nucleoli are larger (or at least some of them are) than in the smaller nuclei. In a given nucleus there may be either (1) from two to four larger nucleoli and a number of smaller ones; or (2) a single large nucleolus and several much smaller ones. In the smaller nuclei the nucleoli are more equal in size than in the larger ones. The largest nucleoli in a nucleus are as a rule of oval or spherical form, with regular contour (an exception is seen in Fig. 26); the irregularly lobular nucleoli (Figs. 23, 25, 27, 28, 33) are usually of medium or small size. There is no apparent regularity with regard to their distribution in the nucleus. None of the nucleoli appear to have limiting membranes.

All these gregarines were fixed with alcoholic solution of corrosive sublimate. With the double stain, haematoxylin and eosin, the larger nucleoli were stained with a deep blackish red, the smaller ones either of the same color or a clearer red; all became stained so intensely by this method that the vacuoles in them were greatly obscured (Figs. 27 and 28).

The Ehrlich-Biondi method produces a yellowish brown or reddish stain of the nucleoli, differences of stain being observable in the different nucleoli of the same nucleus (Figs. 26, 31–35). This staining method brings out very clearly the vacuoles in the homogeneous (?) ground substance of the nucleolus; the structureless substance of these vacuoles stains less intensely than the enveloping substance. Vacuoles are absent in the smallest nucleoli, as well as in those of irregular form; in the larger ones they are almost invariably

present, though variable in size and number. They do not regularly arise at one particular part of the nucleolus, as we found to be the case in the preceding species. Further, there is rarely in this species a single large excentric vacuole ; but as the figures show, usually a number are present, either arranged in a circular row near the periphery, or in a row around a larger central vacuole, or grouped together at one point in the nucleolus. There can be no doubt that the larger vacuoles are produced by the fusion of smaller ones, since two or three smaller ones are frequently found in close contact with each other.

The double stain, haematoxylin and alum carmine, gives different results from the preceding stains, in that by it not only the different nucleoli within a nucleus become colored differently, but also in some cases different stains of the different portions of the same nucleolus are attained (Figs. 22–25). It is only the larger nucleoli, those with regular contours, which become differentially stained in this manner. In such a large nucleolus a portion of its substance stains a deep blue (haematoxylin), another portion or portions purplish or reddish (alum carmine) ; the part stained blue is usually central in position, and encircling it is a zone of red-stained substance. In one case (Fig. 22) the two opposite poles of the nucleolus were reddish, the intermediate part being a deep blue. The medium-sized, irregular nucleoli always stain blue throughout, the smaller ones usually red, but sometimes blue. This stain, accordingly, shows that in this gregarine some of the larger nucleoli are composed of two different substances similarly as we had found two substances in the preceding species, though there by using the methylen-blue-brasilin stain.

With all three staining methods employed, a mass of irregular granules is present in each nucleus, which stain less intensely than the nucleoli. In the smallest nuclei (Figs. 22–25) these granules are more or less regularly distributed through the nucleus, but in the larger ones (Figs. 28, 31–35) they compose a dense mass around the nucleoli or around the largest nucleolus, while the peripheral portion of the nucleus remains nearly free of them. Delicate, faintly stained fibers transverse

this peripheral part of the nucleus, which may be radially disposed or else form a loose network. The size of the granules, their abundance and staining intensity vary in different nuclei of the same size, and there is no sharp distinction between the smallest nucleoli and the largest of these granules. In this species, as in the preceding, I was unable to detect any substance which stained like chromatin.

I have been unable to determine the origin and ultimate fate of these nucleoli, owing to lack of material ; but a few justifiable conclusions may be drawn from the facts at hand. Thus the number and size of the nucleoli stand, as a rule, in a direct ratio to the size of the nucleus. Further, those irregularly lobular nucleoli described above probably represent amoeboid changes of the nucleolus, such as have been seen in life by previous investigators, though it is strange that these nucleoli differ from all others in consisting of a single substance and in containing no vacuoles. Lastly, the number and size of the vacuoles increase, as a rule, with the size of the nucleus.

It is worthy of mention that usually there are a larger number of very small nucleoli in the larger nuclei than there are in the smaller nuclei, although the largest nucleoli of the former are much larger than the largest nucleoli of the latter nuclei. We must conclude, then, that though the size of the nucleoli increases as a rule with that of the nucleus, new nucleoli are also being formed as the nucleus grows larger. Now some of these new small nucleoli found in the largest nuclei have undoubtedly been produced by division from some of the larger ones : thus I have frequently observed irregular (amoeboid) nucleoli with oval prolongations, or with small nucleoli closely apposed to their surfaces, and it probably is correct to conclude that such small nucleoli are in process of division from the larger ones (Figs. 23, 25, 27, 28, 33). Whether all the small nucleoli of the larger nuclei have had such a formation is difficult to determine, since in some of the largest nuclei most of the smallest nucleoli may be peripheral in position, close to the nuclear membrane, and far removed from the larger nucleoli, so that it might seem that the substance of these was extranuclear in origin. The

mass of irregular granules within the nucleus appears to stand in some relation to the growth of the nucleoli, at least there is a relatively greater amount of this substance in the larger nuclei ; it envelops the largest nucleoli and imbibes the same stains, though more faintly, with which the nucleoli become stained. Now as the gregarine grows, at the same time both nucleus and the total mass of nucleolar substance increase in size ; but the nucleus cannot grow without the addition of a substance or substances to it, which have been derived from without. Accordingly, I suppose that the substance of these granules has an extranuclear origin, a substance, *i.e.*, which, having penetrated the nucleus from the cytoplasm, undergoes a chemical change in the nucleus and there becomes precipitated in the form of granules, for no such substance occurs in granular form in the cytoplasm. The growth of the nucleoli might then be explained on the assumption of the intussusception of this substance by the nucleoli. This explanation is offered merely as a hypothesis, since I cannot prove its correctness with the limited material at my disposal. Since no chromatin was demonstrable in these nuclei, it remains for future workers to show whether the chromatin is in these stages commingled with the nucleolar substance, or whether it is represented by one of the two substances of which some of the nucleoli are composed ; and if so, whether all, or whether only a certain number, of the nucleoli are thus partially constituted of chromatin.[1]

### *C.* METAZOA.

#### *a. Egg Cells.*

##### 1. *Montagua pilata* (Verr.).

(Plate 22, Figs. 57–63, 65–87.)

In the germinal vesicles of this mollusc two kinds of nucleolar structures occur : the true nucleolus, which is of large size and almost invariably single ; and certain secondary structures,

[1] For observations of other authors on nucleoli in *Gregarinida*, *cf.* the reviews of Minchin ('93), Van Beneden ('69), Marshall ('92), Frenzel ('93), Koelliker ('49), A. Schneider ('75, '83), Wolters ('91), Carnoy ('84).

which appear at only a certain stage of the cell. The true nucleolus may be considered first, then these other structures, or "pseudonucleoli."

There is always one true nucleolus to each nucleus, and in only two cases out of hundreds of ova examined have I seen two nucleoli (Figs. 57 and 61). The position of the nucleolus within the nucleus is in most cases excentric, seldom central, and never apposed to the nuclear membrane; it apparently lies free in the caryolymph, and is not supported by the chromatin threads. In the youngest, most immature germinal vesicles (I have not studied it in the ovogonia) it is apparently wholly homogeneous, dense, not noticeably refractive, and usually spherical (Figs. 57–61); sometimes, however, it shows an oval or more elongate form, and in the latter case its long axis usually coincides with that of the nucleus (Fig. 58); it is never irregular in outline.

The nucleolus always colors differently from the chromatin, when treated with double stains, as follows:

| Stain. | Nucleolus. | Chromatin. |
|---|---|---|
| Ehrlich-Biondi | maroon | green. |
| Haematoxylin, eosin | orange red | blue. |
| Acid carmine, nigrosine | blue | red. |
| Haematoxylin, fuchsine | purple | blue. |
| Flemming's stain | yellow | violet. |

With the increase in size of the nucleus the nucleolus enlarges, and in such a way that the size of the latter usually preserves its proportion to that of the former; but as the figures show, this proportion is quite frequently not preserved. What may be termed the *first stage* of this nucleolar growth consists merely in an increase in the amount of the homogeneous substance, and between the largest homogeneous nucleoli (Fig. 65) and the smallest (Fig. 57) there is no difference except one of size.

The *second period* of nucleolar growth is introduced when vacuoles commence to appear in the substance of the nucleolus (Fig. 62). Since my observations show that these nucleolar vacuoles are derived from small fluid globules which first appear

in the nuclear sap, these globules may best be treated first. In the nuclear sap, at a certain stage in the growth period of the germinal vesicle, small globules of varying size occur ; there are usually one or two of them in a given nucleus, but sometimes they are quite numerous (*Nut. Gl.* in Figs. 62, 63, 69–71, 73, 75, 81). When I first noticed these structures I conjectured that they might represent centrosomes such as have been found within nuclei at stages previous to mitosis (by Brauer in the spermatocytes of *Ascaris*); but further investigation shows that they have no kind of relation to centrosomes, since they vary in number and size, and further they readily imbibe stains, which centrosomes do not. They have a close resemblance to the smallest yolk granules found in the cytoplasm in point of form, size, and manner of staining. However, sometimes one or two of these bodies may be found in the nucleus when there is no evidence of yolk in the cytoplasm. Accordingly, they would seem to consist of a substance very similar to the young yolk at the time of its first formation. And since they may arise in the nucleus before yolk spherules appear in the cytoplasm they are probably not always taken up by the nucleus from the cytoplasm in the form of globules, but acquire this spherical form first in the nucleus. In other words, we may consider that the nucleus assimilates from the cytoplasm a thin fluid, similar to, if not identical with, that from which the yolk spherules themselves are ultimately formed, and that in the nucleus this substance becomes deposited in the form of globules, perhaps after having undergone a chemical change within the nucleus. Further, this substance must be regarded as having a nutritive value, on account of its similarity to the substance of the yolk, which certainly is nutritive in function. In the more mature, larger germinal vesicles (Fig. 78) large yolk globules are usually found, and are wholly similar to those in the cytoplasm in these stages ; as can be easily determined, their position within the nucleus is not due to removal by the knife in sectioning, so that as the nucleus becomes larger it regularly takes up large yolk globules from the cytoplasm, and from these probably derives the greater part of the nourishment necessary for its rapid growth. We

may conclude, then, that when the nucleus is comparatively small, and when no yolk or only small yolk globules are present in the cytoplasm, the nucleus derives a nutritive substance from the cytoplasm, which is closely similar to that composing the youngest yolk globules ; but when the nucleus has grown large, and the cytoplasm is packed with large yolk globules, it has the power to take up these larger globules also.[1]

To return, then, to the second stage of nucleolar differentiation. This stage does not commence when the nucleolus has attained a certain size, but may commence in some nucleoli earlier than in others; and again it is not marked by a particular stage of development of the yolk in the cytoplasm. The fluid vacuoles probably stand in a genetic relation to the small nutritive globules found in the nucleus, which have been just described. That is, these globules of the nucleus penetrate into the nucleolus and then constitute the fluid vacuoles of the latter structure. I have reached this conclusion after observing that the vacuoles of the nucleolus and the small nutritive globules within the nucleus always stain in exactly the same way. This assumption is further strengthened by the fact that, when the nutritive globules lie in the nuclear sap at some distance from the nucleolus, they have invariably a spherical form ; but in those numerous cases where they may be seen apposed to the outer surface of the nucleolus they become flattened against the surface of the latter, as if the nucleolus were (figuratively speaking) a loadstone which attracts them to itself (Figs. 63, 69, 75). If this origin of the vacuoles of the nucleolus were not the true one it would be difficult to explain their mode of genesis, since there appears to be no other substance within the nucleolus from which they could be derived, and there is no reason for supposing that the

[1] The intensity in the staining of the yolk globules increases with their size, and the largest stain much more deeply than does the nucleolus. During all the earlier growth stages the nuclear membrane is retained, and it is seldom, and then only slightly, irregular in outline; therefore the yolk cannot be taken up by the mechanical aid of amoeboid processes of the nucleus, but its substance must osmotically penetrate the nuclear membrane. And as I mentioned above, it does not seem probable that the yolk globules retain their shape while penetrating this membrane, but diffuse through it in the form of an irregular fluid mass, and then in the nucleus this fluid becomes re-formed into globules.

substance of these vacuoles is a differentiation of the nucleolar ground substance. We may assume, then, that this explanation of the genesis of the nucleolar vacuoles is the correct one, and now proceed to explain the changes in the nucleolus during the successive development of its vacuoles. If we take the size of the nucleolus as a general criterion (though it is not an infallible one, since there are considerable individual differences in different nucleoli (*cf.* Figs. 62, 65, 80)) of the stage of the nucleolus, the process of assimilation of the nutritive globules from the nucleus by the nucleolus seems to be in general as follows : first, one or two globules are taken into the nucleolus, and later when others (apparently a varying number) are also taken up into it, we reach a stage when the nucleolus contains a number of fluid vacuoles (the assimilated nutritive globules) (Figs. 64 and 70). Then these vacuoles commence to fuse together (Figs. 63, 66, 72), finally by their fusion giving rise to one large vacuole, which fills about three-quarters of the space of the nucleolus, and always lies excentrically within the nucleolus (Figs. 68, 69, 73, 77, 79). The nucleolus has now attained its greatest dimension and is either perfectly spherical, or more usually ovoid in shape. Its large excentric vacuole is encircled by a peripheral layer of the primitive homogeneous ground substance of the nucleolus, which has undergone no structural or chemical change. This layer of ground substance becomes necessarily thinner as the vacuole becomes larger, *i.e.*, as the pressure from within becomes greater. But since the large vacuole lies peripherally, the peripheral substance of the nucleolus remains thickened at that point opposite the vacuole, and this thickened portion of the nucleolar wall has most frequently the form of a concavo-convex lens (or on a cross-section, of a half moon), the concave side of which borders upon the vacuole. This thickened part, as the remaining portion of the peripheral layer of the nucleolus at this stage, is in every respect identical with the ground substance of the nucleolus in earlier stages, before vacuoles had made their appearance in it ; and the total amount of the substance of the peripheral layer seems to be equal to the amount of the homogeneous substance of the nucleolus at the end of the preceding

stage. Accordingly, in this second period of the nucleolar growth there appears to be no increase in the amount of the true nucleolar substance, but merely an increase in the amount of the vacuolar substance. The thickened portion of the peripheral layer of the nucleolus is at first biconvex, but as the large vacuole grows larger the pressure of the latter causes it to gradually assume a concavo-convex form (Figs. 84–86). Thus the shape of the large vacuole is at first concavo-convex, and later spherical or oval. This thickened portion of the outer layer of the nucleolus is usually homogeneous in structure, as is the remainder of the true nucleolar substance which envelops the vacuole ; but sometimes small vacuoles may occur within it also (Fig. 71).

Two poles may be distinguished in the nucleolus at this second stage of its differentiation : (1) the pole at which the large vacuole lies ; and (2) the pole at which the thickened mass of the true peripheral substance is situated. From the study of a large number of nuclei at this period I find that in about 75% of them the second of these poles is directed towards the nuclear membrane, the first pole towards the center of the nucleus ; at this stage, as in the preceding, the nucleolus lies usually excentrically within the nucleus.

The later differentiation of the nucleolus consists, accordingly, in the accumulation in it of fluid vacuoles (their substance identical with that of the nutritive globules of the nucleus), but the true nucleolar substance undergoes no change whatever, as far as can be determined from differential staining. There is no chemical union of the vacuolar with the true nucleolar substance, but the fluid vacuoles simply push aside this substance, so that, after these numerous smaller vacuoles have united to form a single large vacuole, the true nucleolar substance remains unchanged as a peripheral layer around this vacuole. The substance of the vacuoles becomes colored with the same stains, though always more lightly, as does the true nucleolar substance, so that we find in this stage a more deeply staining envelope of substance around a less deeply stained portion. This difference of staining between these two parts of the nucleolus is best shown by employing haematoxylin

and eosin (Figs. 68 and 69). With the Ehrlich-Biondi method this difference is not quite so clearly demonstrable. The latter stain is peculiar and differs from all other stains used by me for these cells, in that it very often gives to the smaller vacuoles of the nucleolus the appearance of black, refractive granules ; but a careful focusing of these supposed granules shows them without doubt to be vacuoles, their apparent solidarity being probably due to the refraction of light by the enveloping nucleolar substance.

The chief result derived from the foregoing observations is that the nucleolus takes up some or all of those nutritive globules which lie in the caryolymph, and whose substance had been probably derived from the cytoplasm. Some of these globules then become collected within the nucleolus, representing its fluid vacuoles ; and these globules, increasing in number at the same time, gradually fuse together and thus give rise to a single large excentric vacuole, which is enveloped by the unchanged true nucleolar substance. Since the substance of these small globules is probably nutritive in function, the nucleolus in thus collecting some or all of them would appear to act as a reservoir for nutritive substance, or as a reservoir for that portion of the nutritive substance accumulated in the nucleus, for which the nucleus may have no use. Of course it is not *a priori* impossible that these globules may represent waste products of a nutritive substance, so that the nucleolus might here fulfill the office of an excretory organ. But the function of these nucleoli can only be decided when the behavior of the nucleolus during the pole-body mitosis is known ; I had no ova showing pole-spindle formations.

Finally, the true nucleolus appears not to be bounded by a special membrane ; after staining with acid carmine and nigrosine the nuclear substance appears bluish green and a red membrane seems to envelop it (Fig. 80), but this appearance is probably due to the refraction of light, since nothing of the kind can be found after the use of other staining methods.

We now come to speak of what I have called the "pseudonucleoli," but merely in order to distinguish them from the true nucleolus, and without wishing to express by the use of

this term any particular significance of these bodies. In eight individuals of *Montagua* which were sectioned, and which were of slightly different sizes, though the various growth stages of the ova were more or less the same in all, in only four were pseudonucleoli to be seen, and in only one of these four were they quite abundant, occurring in about 30% of the larger germinal vesicles. There are never more than from one to three in a nucleus. They are usually irregularly spherical and sometimes even angular in form (*Ps. n.* in Figs. 72–77, 79). The largest attained about three-quarters the size of the true nucleolus (of the same nucleus), though this size was attained by few, since they are, as a rule, but little larger than the nutritive globules which are observed in the caryolymph. Each pseudonucleolus consists of a denser, more deeply staining layer surrounding a less dense, more faintly staining core. The denser outer layer is homogeneous, somewhat refractive, and stains in the same manner as the ground substance of the true nucleolus. In smaller pseudonucleoli this outer portion appears on cross-section as a deeply staining ring, with regular outlines, but in the larger ones small, irregular prominences may often be seen on its inner surface. The peripheral layer or ring, further, shows a double contour, but I am unable to determine whether it is bounded by an outer membrane. It increases slightly in thickness with the growth of the pseudonucleolus, and in one case (Fig. 77) it was noticeably thickened at one pole, which gave to it somewhat the appearance of the *tout ensemble* of a true nucleolus. This peripheral layer surrounds a homogeneous, non-refractive, probably fluid mass, which either stains not at all or else only faintly ; when it stains, it is either in the manner of the caryolymph or of the vacuoles of the true nucleolus. I have never noticed that the nutritive globules of the nuclear sap were apposed to these pseudonucleoli. What their origin is, and what their relation to the true nucleolus, I do not know. They are never found in contact with a true nucleolus and so are probably not buds from one. It is curious that they were frequent in the ova of only one mollusc, and in the same stages of the eggs of three other individuals were present in only a few cells, and in four other individuals were

present in none of the ova, though here the same stages of the ova were present as in the first individual. When they occur it is only in the larger germinal vesicles. They are apparently structures *sui generis*, and I have only the suggestion to offer, that they might be characteristic of a particular generation of egg cells, as their absence in the ova of some of the individuals of the mollusc would render probable (compare the observations of Häcker, '93a, where nucleolar differences were found in the ova of primiparous and multiparous individuals of *Cyclops strenuus*).

In Fig. 70 is a remarkable case depicted, namely, two small nuclei lying within a larger germinal vesicle, the former having apparently been assimilated by the latter.

### 2. *Doto.*

(Plate 22, Figs. 64, 68, 69.)

The nucleolar differentiation of these ova is essentially as in *Montagua*, so that no detailed description of the process need be given here. But in the five individuals of *Doto* which were sectioned, no traces of pseudonucleoli were seen, and the nutritive globules within the nuclear sap are usually smaller and much more numerous than in *Montagua*. The yolk globules also have different shapes in these two genera.[1]

### 3. *Amphiporus glutinosus* (Verr.)

(Plate 24, Figs. 140–158.)

(For descriptions of the connective-tissue elements of the nemerteans, from which the genital products are derived, *cf.* my previous paper '96.)

In the nuclei of the connective elements, by a differentiation of which the ova are produced (without any intervening

[1] For the observations of other authors on molluscan germinal vesicles, *cf.* the reviews of the papers of Wagner ('35, '39), Flemming ('74), O. Hertwig ('78b), Lönnberg ('92), Balbiani ('65b), Platner ('86), Leydig ('55a, '50), Stauffacher ('93, '97), Stepanoff ('65), Lovén ('49), Mark ('81), List ('96), Blochmann ('82), Trinchese ('80), Heuscher ('93), Hubrecht ('81), Carnoy ('84, '85), Wirén ('92), Fol ('89), Lacaze-Duthiers ('57), Quatrefages ('49).

mitosis), I could find no nucleoli ; but one or two small minute nucleoli might nevertheless be present within these nuclei, but escape detection, owing to their small size and to the comparatively great amount of chromatin. These nuclei are usually elongated and irregular in form (Figs. 144 and 145, *C. T. N.*).

The smallest germinal vesicles, which are recognizable as such by slightly larger dimensions and more regular, spherical shape, show likewise no recognizable nucleoli.

In what may be termed the *first nucleolar stage*, the nuclei have grown still larger, and in them are to be seen from one to about twelve small nucleoli. These are all peripheral in position, being flattened against the inner surface of the nuclear membrane, which results in their not being spherical, but more or less flattened, lens-shaped, or hemispherical (Figs. 140 and 141).

*Second nucleolar stage.* — The peripheral nucleoli commence to wander towards the center of the nucleus, at the same time growing larger and increasing in number (Figs. 142–145, 152). This process goes on until a considerable number of quite large nucleoli are present, none of which are any longer in contact with the nuclear membrane. As a rule they are not evenly distributed throughout the nucleus, but groups of them occur at different points in the nucleus (Figs. 153, 146–150). This period of differentiation, then, consists in the grouping of most or all of the nucleoli at or near the center of the nucleus, accompanied by their increase in size. There is no ground for supposing that at this stage they fragment into smaller nucleoli ; but very frequently groups of two or three nucleoli may be seen in close contact with one another, and these would represent states of fusion rather than of division, since they are found to be flattened at the point of contact, and not attenuated. Thus the increase in the size of the nucleoli would be due, in part at least, to fusion of contiguous ones. While some of the nucleoli have left the periphery of the nucleus, others are at the same time forming there, which in their turn eventually reach the center, so that a continual process of formation of nucleoli, and wandering of those already formed towards the center, takes place at this stage.

*Third nucleolar stage.* — The nucleoli increase in number, but gradually become smaller and wander towards the periphery of the nucleus (Figs. 154 and 155), until they all lie close to the inner surface of the nuclear membrane. In this stage they attain their maximum staining intensity, as is well seen after the use of Heidenhain's iron haematoxylin, by which they become colored a greenish blue (Fig. 157), while in the previous stages they are brownish yellow, unstained by the haematoxylin.

*Fourth nucleolar stage.* — Vacuoles of varying size arise in the nucleoli, and become somewhat irregular (instead of spherical) in outline (Figs. 156 and 158). In numerous nuclei it may be noticed that all the nucleoli lie close to the nuclear membrane, except a single one, which is placed nearer the center and differs from the others in not staining with haematoxylin, though it usually contains vacuoles ; it may be a nucleolus which has not developed as fast as the others have (Fig. 156).

All nucleoli in the third and fourth stages are very uniform in size, and smaller and much more numerous than in the second ; since there are no facts which permit us to conclude that new nucleoli are being formed in the last two stages we must consider that in them a division of the nucleoli must take place, and this would explain their increase in number and concomitant decrease in size. The fourth stage would seem to be characterized by the commencement of a degeneration of the nucleoli, if the presence of vacuoles and the irregularity of form may be taken as a criterion of degeneration. Neither in this species nor in the other nemerteans examined have I seen stages showing the formation of the pole spindle, so that I cannot describe the ultimate fate of the nucleoli. But the observations of those who have studied these divisions seem to show that they all disappear before the pole spindles are produced; and accordingly the phenomena characteristic of our fourth nucleolar period might represent the commencement of these degenerative processes.

The method of formation of the yolk may next be considered, since the yolk stands in a certain relation to the genesis of the

nucleoli. The cytoplasm, when the yolk first arises in it, stains with haematoxylin (with the double stain of this and eosin); this blue stain of the cytoplasm I have noticed to be characteristic for the cytoplasm of many immature ova, while the cytoplasm of somatic cells usually stains with eosin. The yolk first appears in the form of large yolk balls (Figs. 144 and 145, *Yk. Bl.*), as they may be termed; the number of these balls varies in cells of the same size, as well as in those of different dimensions, and they appear to be produced successively in a cell, until at the end of the third nucleolar stage they all have disappeared, having given place to the mature yolk spherules. They arise in the cytoplasm at no fixed point, though usually at some distance from the nucleus; it is hardly necessary to state that they stand in no genetic relation to the nucleus, either in this or in the other nemerteans studied. The yolk balls are at first dense and homogeneous, and stain intensely with eosin; the size that they may attain while still homogeneous is very variable. Subsequently they become vacuolated, even sometimes granular, and different portions of the same ball may stain differently, which shows that both a chemical and a physical change takes place in their substance. Finally, they fragment into unequal sized granules, which stain less deeply, and then these latter split up further, until the ultimate yolk spherules (*Yk. Gl.*) are produced. In the largest ovarial eggs all the yolk balls have disappeared (they linger longest at the periphery of the cell), the cytoplasm being densely filled with the yolk spherules. In some cases yolk balls lie in the cavity of the gonad (Fig. 155), and these are probably derived from degenerated ova.

The following facts show, I think, that the nucleoli stand in a genetic connection with the yolk substance. The nucleoli stain in the same way and have in other respects the same appearance as the smaller fragments of the yolk balls and as the mature yolk spherules (Figs. 144–146). Fragments of yolk balls occur frequently in close contact with the outer surface of the nuclear membrane. Now since the nucleoli first appear in contact with the inner surface of this membrane, the conclusion is plausible that the nucleoli represent portions of

a yolk substance, either of the yolk-ball fragments or a substance equivalent to that out of which the latter are differentiated, and this substance, then penetrating osmotically the nuclear membrane, becomes deposited or precipitated in the nucleus in the form of spherical globules, which are the nucleoli. From this yolk substance taken into the nucleus the chromatin, linin, and nuclear sap might derive the nourishment necessary for their growth, and those nucleoli which remain through the fourth nucleolar stage might represent either a reserve supply of this nourishment, or chemically changed portions of it, from which all nutritive substances have been extracted ; the latter view would seem substantiated by the fact that the nucleoli stain somewhat differently in the third and fourth stages.

The nuclear membrane is present during all these stages. The nucleus is always regular in outline, usually oval, except during the third stage, when it may become slightly irregular, though it never becomes noticeably lobose or amoeboid.

In the first nucleolar stage (Figs. 140 and 141) the chromatin appears as a network of delicate fibers, which stain with haematoxylin. Towards the end of the second stage (Figs. 146–150) it assumes the form of irregular masses, and the fibers become less numerous. In the largest ovarial nuclei (Figs. 154 and 157) it is finely distributed throughout the nucleus in the form of minute microsomes ; traces of fibers may be found only at the periphery of the nucleus, though I have not determined whether these are fibers now for the first time forming, as is the case in the other nemerteans. The nucleoli are never suspended by the chromatin fibers.

This species is characterized by the formation of a membranous structure in the cytoplasm, during the second and third nucleolar stages, which is present in none of the other nemerteans. This is a membrane within the cytoplasm, separated from the nucleus, as well as from the cell membrane by cytoplasm ; it lies close to the nucleus (Figs. 146 and 155, *Iv. Mb.*). It is thicker than the nuclear membrane, though not so dense, and differs in no wise structurally from the cytoplasm, except in its greater density, the cytoplasmic granules in it lying closer together (these granules appear to be

the nodal points of a "Wabenwerk" in the sense of Bütschli). This intracellular membrane is not open at any point, and a longitudinal section of it shows it to be not spherical but oval in outline, the apices of the oval being furthest removed from the nucleus. It is present only in the second stage of the nucleolus, and between it and the nucleus no yolk balls occur. I have never seen such a structure in any other egg cells except in the ova of *Gryllus abbreviatus;* a similar structure was found by van Bambeke ('**83**, eggs of *Leuciscus*, *Lota*), Shäfer ('**80**, egg of *Lepus*), and Gerould ('**96**, *Caudina* egg).

4. *Tetrastemma catenulatum* (Verr.) Montg.

(Plate 23, Figs. 103–133; Plate 24, Figs. 137–139.)

The formation of the yolk may be spoken of first, then the nucleoli proper, and afterwards certain large nuclear structures which may or may not represent nucleoli of another kind.

The yolk first appears in the form of one or two yolk balls (*Yk. Bl.*, Figs. 107, 108, 112, 114–116) in the cytoplasm; the larger ones are regularly oval as a rule, and the smaller ones spherical. A number of these yolk balls are produced successively in each cell, and by their fragmentation the ultimate yoke spherules (*Yk. Gl.*) are evolved. Each such ball is at first smaller than the nucleus of the cell in which it occurs, but gradually increases in size, though the maximum size which it may attain is not a fixed quantity, but is quite variable. As it increases in size it also gradually becomes more deeply stained, attaining its most intense staining when it has attained the limit of size. The substance of these balls is dense, finely granular, not brittle, somewhat refractive; in the youngest stages of their formation they often appear nearly homogeneous. About the time a ball has reached its maximum size it commences to change both structurally and chemically, vacuoles appear in it, it begins to stain less intensely, and becomes irregular in outline. Thus it becomes either coarsely granular, or else unstaining vacuoles appear scattered through it, and with eosin stains no longer a deep red, but a light red or even yellowish. Next it breaks into a number of pieces, whereby

the primitive yolk ball may break either into two fragments (which are usually unequal in dimensions), each of which then fragments further, or it breaks at once into a considerable number of larger granules. The final stage in this process of division shows the daughter yoke balls fragmenting to form the ultimate yolk spherules (Fig. 118); the latter stain an orange red with eosin, are homogeneous in appearance, and usually oval or spherical in form, seldom irregular. Two main stages may accordingly be distinguished in the formation of the yolk: (1) the formation of a large, regularly shaped yolk ball; and (2) the successive fragmentation of this ball, accompanied by a gradually lessening affinity for stains, resulting in the evolution of the mature, small yolk spherules, the cytoplasm of the ripe egg being thickly filled with the latter. It is usually the case that the yolk ball attains its greatest size at the end of the first stage. In cells of medium size all the various stages of yolk formation may be found, which shows that the yolk balls are being successively produced and are successively fragmenting; quite a number of these balls need to be produced in order to furnish the large quantity of yolk globules of the mature egg. The time when the yolk balls first appear, the size they reach, and the manner in which they segment, seem to vary much in individual cells.

I have not been able to determine the manner of the first differentiation of the yolk substance in the cytoplasm. Two possible explanations suggest themselves: (1) either a certain portion or constituent of the cytoplasm changes into yolk substance; or (2) the yolk balls may represent a nutritive substance accumulated in the cytoplasm, which may have been derived from the blood or from some neighboring tissue, if not directly from the posterior intestine. But it is without doubt that this substance is not of nuclear origin, for the yolk balls at their first appearance are not in contact with the nucleus, but usually at some distance from it; and also during the earlier stages of the yolk formation the nucleus is irregular in outline, with short, blunt processes, which would show that it is taking up substances from the cytoplasm, rather than excreting substances.

The cycle of the formation of the nucleoli may here also be divided into three stages, which do not quite correspond to the four of *Amphiporus gelatinosus*.

*First nucleolar stage.* — In the smallest germinal vesicles found one or two relatively very large nucleoli were present, one of them often in the center of the nucleus, the other more excentric or even against the nuclear membrane (Figs. 103, 114, 115). The nucleoli in these smallest nuclei are as large or nearly as large as in any of the following stages. In germinal vesicles of slightly greater dimensions three or four nucleoli may be present, and some of these may have increased a little in size ; the amount of nucleolar substance at this stage is often so great as to occupy a fifth of the nucleus. They now increase in number, until at the close of this period we find a considerable number of mostly large nucleoli quite evenly distributed through the nucleus (Figs. 104–106, 109, 110, 116), but often they are at one of its poles more numerous than at other points. This stage would seem to correspond to the first and second of *Amphiporus glutinosus*.

*Second nucleolar stage.* — The nucleoli continue to increase in number but now decrease in size and commence to pass to the periphery of the nucleus, until at the end of this period they all lie close to the nuclear membrane, are regular in outline, and adequal in size (Figs. 107, 119, 122, 124–126, 130, 131). At the beginning of this stage numbers of nucleoli may be found arranged in chain-like rows, as is to be seen in Fig. 111. This would correspond to the third stage of *Amphiporus*.

*Third nucleolar stage.* — Nearly all the nucleoli are close to the nuclear membrane, often flattened against it (Figs. 117, 120, 127, 129, 137, 138). They show signs of degeneration; thus they stain less intensely, are irregular in outline, and have a vacuolar or granular structure. In the largest germinal vesicles their number has apparently decreased and small non-coherent masses of granules may be seen, which are probably degenerated nucleoli. Sometimes a nucleus may be found in this stage in which almost all of the nucleoli contain each one large, excentric, lightly stained globule or vacuole (Fig. 117).

*Staining of the nucleoli.* — The natural color would appear

to be a light yellow. In a preparation stained with haematoxylin and eosin, though not very thoroughly colored with the latter stain, the large nucleoli of the first nucleolar stage were of a light-yellow color, apparently stained only slightly with the eosin; those of the end of the second stage were mostly stained red, and those of the third stage were stained red, except those which had broken into granules, these latter being stained very little. In another preparation, in which the eosin had acted for one or two minutes longer than in the preceding preparation, the nucleoli in the first stage were stained orange, those of the second stage red, and those of the third stage very slightly or not at all stained. Accordingly, they stain more lightly at the commencement of the first and at the end of the third stage than during the second stage; these differences of stain are probably due to chemical differences in the nucleoli at different stages.

The chief differences between the nucleoli of this species and those of *Amphiporus glutinosus* are as follows: in the former there is no stage which exactly corresponds to the first stage of the latter, where we found a number of small peripheral nucleoli; in *T. catenulatum* there are at first one or two large nucleoli which are not always peripheral in position. The nucleoli in the third stage of *T. catenulatum* are more irregular in form and dimensions and stain less intensely than those of the fourth stage of *Amphiporus*. But the most important difference between the two species is to be found in the fact that in *T. catenulatum* new nucleoli continue to be produced even in the third stage. Thus there are at the periphery of the nucleus, between the larger degenerating nucleoli which had their origin during the first stage, also much smaller, newly formed nucleoli arising while the former are disappearing. Such younger nucleoli may be seen at the close of the third stage, when the nuclei are largest and chromatin filaments appear in them, arranged in contact with the chromatin threads or near to them (Figs. 127, 137, 138). These smallest nucleoli of the third stage always stain intensely red with eosin, while the much larger ones of the first and second stages stain more of an orange color with this stain. This difference of staining in these two kinds of nucleoli might be explained thus:

As we had concluded for the preceding species, so also in the present and in the species of nemerteans yet to be described, the nucleoli are in all probability accumulations within the nucleus of a substance taken up from the cytoplasm, this substance being related to that which constitutes the yolk balls. In the least mature germinal vesicles of *T. catenulatum* we found one or two very large, lightly staining nucleoli; these stain in the same way and show the same structure and degree of refraction as do the daughter yolk balls (Figs. 107 and 116). Further, I have noticed in the cytoplasm small yellowish spherules (yolk-ball fragments) which are in every way similar to the smaller nucleoli, and quite frequently I have observed one or two of them pressed so close against the outer surface of the nuclear membrane as to cause a depression of the latter (Figs. 112 and 118). In other words, it would seem that the substance of some of the yolk-ball fragments is taken into the nucleus and in the latter is re-formed into nucleoli. As long as yolk balls or their fragments are found within the cytoplasm lightly stained nucleoli of approximately the same dimensions as these may be seen in the nucleus. I have never seen a pore in the nuclear membrane through which a yolk-ball fragment could penetrate, though this membrane sometimes appears to be thinner at the point of contact with a yolk-ball fragment than at other points in its circumference. But in the third stage, when all yolk balls and their fragments have disappeared and the whole cytoplasm is thickly filled with their derivatives, the mature yolk spherules, large, faintly staining nucleoli, are no longer present in the nucleus, but the smallest nucleoli present at this time resemble in form, size, and stain, the yolk globules. Therefore we must conclude that the young, small nucleoli which first appear about the end of the third nucleolar stage represent mature yolk spherules, or at least that the substance of the two is equivalent. While the nucleoli of the first generation (formed in the first stage) are commencing to degenerate, new nucleoli of a second generation begin to arise in the nucleus, and the latter, which may serve as nourishment for the chromatin threads, differ from the former genetically, in that they are not assimilated portions of yolk-ball fragments, but

assimilated yolk spherules. Thus, as we find in the cytoplasm first yolk balls, then their fragments, and finally the mature yolk spherules, so in the nucleus the first generation of nucleoli are assimilated yolk balls and their fragments, while the small ones of the second generation are derived from the only yolk elements then present in the cytoplasm, namely, yolk spherules. The nucleoli of the first generation also differ from those of the second, at the time of the first appearance of both, in their manner of staining; so that they would seem to differ chemically from each other.

*Nuclear structures of problematical significance.* — In only one out of the three individuals of this worm studied were the following remarkable structures to be observed, though the fixation method of both of the other individuals was exactly the same. These bodies first appear in ova of the second nucleolar stage, but here show always the same typical structure, so that I can say nothing as to the manner of their first formation. In preparations stained with haematoxylin and eosin they are colored by the former stain a little more deeply than the nuclear chromosomes, so that they stand out sharply in the nuclear substance (*N. Bd.*, Figs. 122–139). The smaller ones, *i.e.*, those of the younger germinal vesicles (Figs. 122–126), are finely granular, though whether they each consist of a mass of fine granules or of homogeneous ground substance in which granules are distributed, I cannot determine. In the larger nuclei they often appeared wholly homogeneous (Fig. 132). In shape they are usually nearly spherical, with a sharp outline, which may or may not represent a limiting membrane; the larger ones are often more irregular in form (Figs. 132, 133, 139). In the smaller nuclei they are as a rule, but not always, smaller than in the larger ones; in the smallest nuclei in which I have found them there is only one of these bodies to a nucleus; while in the larger nuclei they are not only larger, but also there may be from one to four of them in each nucleus. In only one small nucleus were three of them present (Fig. 128). In two cases, both larger nuclei, I found division stages of these bodies: in the one case (Fig. 131) the body was ovoid in outline, with a shallow constriction at right angles to its

longitudinal axis, at about its middle ; in the other case (Fig. 129) the body was plainly biscuit-shaped, with a well-marked medial constriction : these would probably represent respectively successive stages of division.

The various stages found would show the metamorphoses of these structures to be as follows : in the medium-sized nuclei, those in which they first appear, there is only one to a nucleus. This one increases in size up to a variable point, when it begins to divide, producing two daughter-bodies, which are not always of equal size. One or both of these bodies may now divide again, resulting in the formation of (respectively) three or four bodies. Since, however, the four bodies sometimes found in the larger nuclei are often quite unequal in size, we must assume : (1) either that the divisions have been very unequal, and each daughter-body had divided ; or (2) that after the first division, which may or may not have resulted in unequal daughter-bodies, only one of the latter divides further, and it divides once, and one of its products divides once. It is to be noted that the number, the size, and the time of the division of these bodies stand in no regular relation to the size of the nucleus. Thus in one small nucleus (Fig. 128) three were already present, so that here two divisions must have taken place; while in some much larger nuclei (Figs. 130 and 133) a single, much larger one was present, which showed no signs of division. In the larger nuclei these bodies are often quite irregular in form ; may this increasing irregularity portend an on-coming dissolution or other degeneration? They were found, as remarked above, in the ova of only one of the three individuals of this species examined, though in all three individuals the stages of egg development were very much alike; in the single individual in which they occurred they were not present in all the larger eggs. Their whole appearance and consistency show that they are not artefacts (the fixation was with hot aqueous corrosive sublimate), and they have no resemblance to any parasitic organisms, as *e.g.*, *Protozoa*, with which I am acquainted. Nor can they be centrosomes nor true nucleoli, and stand in no apparent relation to the nucleoli. In a single case I found two nucleoli enclosed by one of these bodies ;

but in no other cases were these structures in contact with nucleoli. They are also never in contact with the nuclear membrane. Male pronuclei they cannot be, since the fecundation takes place in later stages than those which I have had opportunity to observe. I must conclude, though with reserve, that they are either parasitic *Protozoa*, or, more probably perhaps, structures which characterize ova of a certain generation. (Compare my remarks on the "pseudonucleoli" of *Montagua*. The structure figured by Henneguy ('93), in the immature germinal vesicles of *Sygnathus* may have some connection with these bodies.)

*Chromatin.* — The chromatin in the youngest germinal vesicles (Figs. 103–105, 112–114) is distributed throughout the nuclear sap in the form of minute microsomes. In the second and sometimes the first nucleolar stage such microsomes can often not be detected, but the whole nuclear substance, with the exception of the nucleoli, appears homogeneous and stains with eosin a yellowish red (Fig. 115). This peculiar coloration might be accounted for on the ground that in these stages there is a diffusion of nucleolar substance throughout the nucleus. Towards the conclusion of the second and the commencement of the third nucleolar stage, the minute chromatin microsomes again become evident (Figs. 118 and 130). At the end of the third stage a few chromatin threads begin to arise in the nucleus (Fig. 127), and these stain slightly with haematoxylin in the same manner as the microsomes do; they appear to arise separately and at different points in the nucleus, and are at first short, but gradually increase in length. As noted above, the small nucleoli of the second generation are often apposed to these threads, and sometimes lie in the meshes of them.

*Nucleus.* — In the first and second nucleolar stages the nucleus has often short, lobular processes, which may be amoeboid in life (Figs. 109, 112, 114, 116, 125); these changes in the form of the nucleus no doubt stand in a direct relation to the assimilation of yolk substance from the cytoplasm. Towards the end of the third stage the nucleus becomes regular in outline, with no traces of amoeboid processes; at this stage also

the nuclear membrane has attained its greatest thickness. The thinness of this membrane in previous stages would allow the penetration of nutritive substances into the nucleus from the cytoplasm. The small nuclei from which the germinal vesicles are directly derived, without any intervening mitoses, are irregular in shape, and no nucleoli are to be seen in them (Figs. 108 and 112, *C. T. N.*).

### 5. *Tetrastemma elegans* (Verr.).

(Plate 28, Figs. 282–299.)

Having only two mature individuals of this worm for study, I am unable to give as thorough a description of the nuclear metamorphoses of the egg as was possible for the other nemerteans ; one preparation was fixed with Hermann's fluid, the other with aqueous solution of corrosive sublimate, but the latter had been too deeply stained (haematoxylin, eosin) to allow the study of certain details, as *e.g.*, the cytoplasmic changes leading to the formation of the yolk. Yolk balls were observed in only a few ova, and are much less numerous than in *T. catenulatum ;* it is possible that the development of the yolk in the present species may be as in *Zygonemertes*, that is, the mature yolk spherules may as a rule be directly formed without the interpolation of a yolk-ball stage.

*First nucleolar stage.* — The youngest germinal vesicle, recognizable as such, showed a large nucleolus close to the nuclear membrane (Fig. 282) ; I have seen no smaller nuclei than this one, but would conclude by analogy from the facts in the other metanemerteans that also here all the nucleoli have an extranuclear origin. In slightly larger nuclei (Figs. 283–287) there are from one to three nucleoli, whose size varies considerably with regard to that of the nucleus, as well as to the size of one another. In such cases (Fig. 283) where only two nucleoli are present, one near the center of the nucleus, the other close to the nuclear membrane, the former is probably the older and has left the periphery for the center of the nucleus, while the other is younger and is still in process of formation. These first-formed nucleoli are usually rather large in proportion to

the size of the nucleus, seldom small. It is the rule that in one, sometimes in all the nucleoli, a large unstaining globule is present, which has the appearance of a vacuole (Figs. 284–287, 298); no nucleolus has more than one such globule. Quite often there is only a single large vacuole-containing nucleolus in a nucleus; or there may be from one to six nucleoli, only one of which contains a vacuole, and then the latter is usually the largest; or again, there may be two or three large nucleoli, nearly equal in point of size, each of which contains a vacuole (of course numerous intermediate stages may be found). There is certainly a successive production of nucleoli, but it is difficult to decide whether some of these after leaving the periphery of the nucleus fuse together, or whether some divide into smaller nucleoli. Now it seems probable that those nucleoli which are formed first are usually unequal in size, both in the same nucleus and in different nuclei, as a comparison of the figures shows. And though a gradual fusion of the nucleoli might play some part in the youngest germinal vesicles, nevertheless it would seem more probable that we have to do in these early stages with divisions of the nucleoli, especially since in the following stage they are much more numerous, as well as smaller. Fig. 287, in which three apposed nucleoli are to be seen, may thus represent a division of a single nucleolus. It is not unlikely that the unstaining globule within a nucleolus might aid, if it is not the direct mechanical cause of, such division. This first nucleolar stage is then characterized by the successive formation of a few comparatively large nucleoli at the periphery of the nucleus, and the migration of these towards the center; the presence of large vacuoles within some of the nucleoli is also a criterion of this period.

*Second nucleolar stage.* — We find a group of numerous nucleoli near the center of the nucleus, which are frequently more numerous than in our Fig. 292. At this stage they attain their smallest dimensions, and are approximately equal in size; they are completely homogeneous and contain no vacuoles. The total number of the nucleoli is apparently greater at this stage than at any other.

*Third nucleolar stage.* — This is characterized by an increase

in the size of the nucleoli, a decrease in their number, and the gradual migration of them towards the periphery of the nucleus. At the beginning of this period (Figs. 290, 291, 293, 294), the nucleoli are quite evenly distributed throughout the nucleus; at its close they are mainly peripheral in position, near the nuclear membrane (Fig. 297). The increase in the size of the nucleoli is due, in some part at least, to the coalescence of every two or three neighboring ones, and such juxtapposed groups of two or three nucleoli may be often found (Fig. 294). None of the nucleoli contain vacuoles.

*Fourth nucleolar stage.* — Now we find unstaining globules or vacuoles reappearing in the nucleoli, and there may be either a single large one to each nucleolus, or a number of smaller ones ; the large one is probably formed by the coalescence of smaller ones. Almost all the nucleoli are in contact with the nuclear membrane, often flattened against it (Fig. 299). They have become larger than in any preceding stage, and less numerous, but are now quite unequal in size. This stage may mark the commencing degeneration of the nucleoli, though I have observed no evidences of a commencing fragmentation.

At the beginning of the first stage the nuclear sap never stains ; but at the end of this period, when the nucleoli have become more numerous, it stains very noticeably with eosin (Fig. 286), which would point to a solution of nucleolar substance in the nuclear sap.

### 6. *Zygonemertes virescens* (Verr.) Montg.

(Plate 27, Figs. 236–248.)

*Yolk.* — In only two cases out of the numerous egg cells examined (three individuals of this worm were sectioned) have I seen yolk balls, so that the formation of yolk balls must be regarded as abnormal, if not pathological ; in this species the yolk arises as minute yolk spherules in the cytoplasm (Fig. 246), without (except in the cases noted) a yolk-ball stage being passed through. These minute globules stain at first very faintly, and when they first appear are isolated from one another. There is no given point in the cytoplasm where they

are first produced, but a varying number are formed simultaneously and at different parts of the cell; it is usually, though not always, the case that they first arise at the periphery of the cell at some distance from the nucleus. The mature yolk globules are slightly larger than these and stain somewhat more intensely, which shows that they gradually become denser as they increase in size; in the largest ova these spherules are so abundant that the true cytoplasm is quite obscured (Fig. 247).

*First nucleolar stage.* — In the smallest nuclei found there is a peripheral group of several nucleoli lying close to the nuclear membrane, which are spherical in form (Figs. 236–238).

*Second nucleolar stage.* — The nucleoli have increased in number, and, departing from their original peripheral position, now occupy the center of the nucleus (Figs. 239 and 240). So small are they, and so densely grouped may they become, that at first sight one might be led to suppose that each group of numerous nucleoli was a single nucleolus. In those cases where the nucleus is oval or elongated in form, instead of spherical (the usual case), in the place of a single cluster two are commonly present, or else the single mass or cluster of nucleoli is elongate in shape, its outline being more or less parallel to the contour of the nucleus. The nucleoli in this stage are always more numerous and usually also smaller than those of the previous period; their increase in number might thus be brought about, in part at least, by divisions of the earlier nucleoli.

*Third nucleolar stage.* — The nucleus now is much larger, and the nucleoli begin to wander apart towards the periphery of the nucleus (Figs. 241, 243, 246, 247). I have observed all stages between nuclei containing centrally grouped, small nucleoli and those in which they have come to lie close to the nuclear membrane. In this stage, as in the preceding one, the nucleoli are perfectly homogeneous without vacuoles, and spherical in form. In a few nuclei, however, they appear greatly vacuolated, but these cases are so rare that they must be considered abnormal. At the end of this period they attain their greatest dimensions, though they thereby become some-

what unequal in size. In this stage, accordingly, they increase in size (perhaps by the fusion of contiguous ones (Fig. 242), and decrease in number, whereas in the preceding one the reverse process took place.

*Fourth nucleolar stage.* — Almost all the nucleoli are flattened against the nuclear membrane (Figs. 245 and 248), and they commence to show a vacuolated structure; these apparent vacuoles, which are unstaining globules, when stained by the Ehrlich-Biondi method, whereby only the ground substance of the nucleolus is colored, appear as refractive granules (Fig. 248). At the conclusion of this period the nucleoli become irregular in shape, granular in appearance, stain less deeply, and each finally breaks up into a mass of granules. In this manner they decrease both in number and size.

During the third and fourth stages, while the nucleoli are undergoing the metamorphoses described, a small number of newly formed ones appear in the nucleus, which are of later formation than the others (Figs. 245, 247, *n. 2*). These may serve as nourishment for the chromatic filaments, as in *Tetrastemma catenulatum;* but in the present species I have not observed any distribution of them along these filaments, and further they are numerically scarcer than in *Tetrastemma.*

No yolk is present in the cytoplasm in the first and second nucleolar stages. This fact is easily proved by the use of the Ehrlich-Biondi stain, by which the cytoplasm is stained green, and the yolk substance, when present, a brownish maroon color. Yolk first appears in the third nucleolar stage, and at the commencement of the following stage the whole cytoplasm is nearly filled with it. Further, the nucleoli stain differently from the yolk globules by the use of the stain mentioned. These facts show that the origin of the nucleolar substance is not to be found in the yolk substance proper, but in a cytoplasmic substance from which the latter may later be evolved. That the substance of the nucleoli is extranuclear in origin is shown by the fact that the nucleoli at their first appearance lie in contact with the nuclear membrane (Figs. 236–238), and only later do they take a central position. Though I have seen no nuclei smaller than those figured, which could without doubt be

classed as germinal vesicles, yet it seems so probable that the substance out of which the nucleoli are formed is extranuclear, that I would conclude, *a priori*, that no nucleoli are present in stages of the germinal vesicle much earlier than those which have been here described. Those small nucleoli of a second generation, which are first produced in the third and fourth nucleolar stages, may represent yolk globules assimilated by the nucleus, since in these stages the cytoplasm is filled with such globules.

On the other hand, the yolk cannot be considered as having its origin in nucleoli which have wandered out of the nucleus, since in none of these stages are nucleoli found in the cytoplasm. And if such were the case, one certainly should be able to observe the large nucleoli of the third nucleolar stage in the cell substance, for it is at this period that the yolk first appears. I conclude that the yolk globules have their origin in some substance contained in the cytoplasm, and that the nucleolar substance also has its origin in some cytoplasmic substance. But whether the primitive nutritive substance of the yolk globules and that from which the nucleolar substance is derived are identical, is of course open to question ; however, judging from the similarity in appearance, we might conclude that the primitive cytoplasmic substance was the same in both cases, and especially if we consider, which seems plausible, the nucleoli to represent the nutritive substance of the nucleus, as the yolk globules certainly represent that of the cell body.

In the first nucleolar stage the nuclear membrane is usually very thin, but always perceptible ; in the later stages it becomes thicker. The nucleus is never noticeably irregular or amoeboid in outline. Might this be explained by the absence of yolk balls in the cytoplasm ?

In the second and at the beginning of the third nucleolar stages, the central mass of nucleoli is usually surrounded by a clear space, in which space few or no chromatin microsomes occur, though it may be transversed by a few achromatic fibers (Figs. 239 and 240). This space was found in most of the egg cells of this stage in the three individuals sectioned, though it

may have been produced by the action of the preserving fluid (hot aqueous solution of corrosive sublimate).

### 7. *Stichostemma eilhardi* (Montg.).

(Plate 27, Figs. 213–235.)

The yolk changes may first be delineated, then those of the nucleoli. In my paper on this fresh-water form ('95), I have described the ovogenesis to some extent, and here shall follow it more in detail.

*Yolk.*— The yolk first appears in the cytoplasm in the form of small, more or less spherical masses (Fig. 213, *Yk. Bl.*), which at first stain like the cytoplasm ; but these youngest recognizable yolk balls consist of a substance in which the fine granules (or nodal points of an alveolar structure) are much more densely grouped than in the surrounding cytoplasm. Thus the young yolk ball may be distinguished from the cytoplasm proper by its greater density. A number of these yolk balls appear to arise simultaneously, though in these earliest, as well as in the later stages of yolk formation, a successive production and metamorphosis of yolk balls take place, since in all but the earliest stages of their development yolk balls occur in the cytoplasm in various stages of formation. There is no rule as to the part in the cell at which these balls are destined to arise, for they may be found anywhere between the nucleus and the periphery of the cell ; the fact that they first arise just as frequently at some distance from the nucleus as in its immediate neighborhood shows that they have no nuclear origin. An anabolic and a katabolic series of changes of each yolk ball can be distinguished, and these series of metamorphoses may be described in succession and termed respectively the prophasis and metaphasis of the yolk balls.

*Prophasis* (*Yk. Bl.* in Figs. 217, 218, and the median ones of Fig. 215). — The progressive or anabolic changes of the yolk balls consist in (1) their absorbing protoplasmic stains with great intensity, so that they stand in marked contrast to the cytoplasm ; and (2) in their becoming quite homogeneous in structure, this homogeneity probably explainable by supposing

that a dense condensation of the fine granules of which they are composed takes place. They continue to increase in size, and gradually stain deeper as they do so, until they attain about the dimensions given in Fig. 217 ; but I am unable to determine whether they all reach exactly these dimensions before the metaphasic changes commence. At the conclusion of this period of their development they are large bodies, regularly spherical or oval in outline, and apparently without a limiting membrane ; especially characteristic is their homogeneity and their intense staining.

*Metaphasis.* — These katabolic metamorphoses are introduced when a few unstaining globules arise in the substance of the yolk balls. These globules increase in number and size until the yolk ball assumes a vacuolated appearance (Figs. 215, 217, 228). At the same time its ground substance loses its staining power and no longer stains homogeneously. At the commencement of these changes the yolk ball may even increase somewhat in size, since the substance of the globules is added to it. These changes continue until the yolk ball either breaks up into the mature yolk globules (*Yk. Gl.*, Fig. 235), or first breaks into a varying number of larger pieces, and then each of the latter divides into yolk globules. The yolk globules are usually nearly spherical in shape, and though by no means equal in size are always larger than those of the other nemerteans examined.

During the prophasis each yolk ball is enveloped by a clear, structureless zone of cytoplasm ; but this surrounding zone is usually not noticeable around the larger yolk-ball fragments, and never around the mature yolk globules.

As to the cause of the fragmentation of the yolk balls, I can find no sure explanation from the facts at hand. However, the appearance of the colorless fluid globules within their substance must have an important connection with these katabolic changes, since they characterize the commencement of this period of change. It would seem likely that these colorless globules represent a fluid constituent of the cytoplasm which has actively or passively been taken into the yolk ball, — perhaps from the clear cytoplasmic zone enveloping each yolk ball,—

since the yolk balls increase in size at the beginning of the metaphasis, though there appears to be no increase in their own ground substance. These unstaining globules are at first localized at different points in the yolk ball ; and it would seem probable that their substance later mixes itself with the ground substance of the yolk ball, since this supposition would account for the lessening intensity of the stain of the yolk ball during the metaphases. It would appear less probable that these globules are metabolic products of the true substance of the ball ; however, we have too few facts to enable us to determine which of these is the correct view.

Certain curious structures found in the cytoplasm of an immature worm fixed with Lang's fluid (aqueous corrosive sublimate solution, NaCl, acetic acid) may be mentioned here. In the cytoplasm of a number of ova, in none of which any yolk was present, I found a few small, ring-shaped bodies, which stained with haematoxylin much more intensely than the surrounding cytoplasm (*Yk. Bl.?* Fig. 233). Each consisted of a ring composed of a dense, homogeneous substance, the inner surface of the ring being smooth, but the outer irregularly jagged, this whole ring (as it appears on a section) enclosing an unstaining vacuole or globule. In reality these bodies are spheres, but my description applies to sections of them. These structures vary considerably in size, and sometimes are not spherical but oval, the larger ones usually staining more deeply than the smaller ones. I found them only in some of the ova of this one individual, and nothing of the kind was to be seen in the ova of about twenty other individuals sectioned, which had been variously preserved in picric, osmic, and chromic acids, in simple aqueous solution of corrosive sublimate, and in the fluids of Hermann and Flemming. Accordingly, they must be regarded as artefacts, produced by the action of the acetic acid, which I have long since found to be a very unreliable fixative for the cytoplasmic elements of the nemerteans. It is most probable that they are young yolk balls, to which the acetic acid has given an abnormal appearance. Or might they represent multiple asters, such as have been recently described by Mead in *Chaetopterus?*

*Germinal vesicles, nucleoli.* — In this genus the earliest egg stages are more favorable for study than in the other metanemerteans. In the connective-tissue nuclei from which the germinal vesicles are directly derived (with no intervening cell generations) no nucleoli are present, though this conclusion was possible only after much careful observation. These small nuclei (Figs. 213, 217, 218, 220, 228, *C. T. N.*) are characterized by a relatively thick membrane and by chromatin which is usually granular in distribution, but which may sometimes occur in the form of granular fibers. These chromatin masses might at first sight be confounded with nucleoli, but their small size and irregular contours show that they are true chromatin granules. Further, when these nuclei are stained by the Ehrlich-Biondi method, these fibers and granules always stain with methylen green (chromatin reaction) and not a single one stains with fuchsine (which invariably stains any true nucleoli). Accordingly, what could not be finally proved for the other metanemerteans, though all observations pointed to its being the case there, could be definitely settled for *Stichostemma*, namely, that these connective-tissue cells contain no nucleoli ; in other words, nucleoli first arise in the definite germinal vesicles.

Before proceeding to the description of the egg cells it may be noted that not all the undifferentiated connective-tissue cells within the gonad become germinal vesicles. I have previously ('95) shown that the young gonad is a cell syncytium in which numerous nuclei are unevenly scattered through a mass of cytoplasm, but cell boundaries cannot be seen (Figs. 217 and 218). A few of these nuclei increase in size and eventually become germinal vesicles, and the latter reach maturity not simultaneously but in succession, so that no gonad contains more than one large ovum at a given time. The numerous other nuclei which do not become thus differentiated degenerate, and their substance is eventually absorbed by the gradually increasing mass of cytoplasm of one of the growing egg cells. These regressive processes are as follows (Fig. 218, *C. T. N.*) : the nuclei increase a little in size, but become much clearer in appearance, *i.e.*, the relative amount of their chromatin appears to decrease ;

next the cell membrane gradually disappears; then the chromatin granules no longer become colored by any of the stains employed, but become refractive and yellowish. All the chromatin granules do not lose their affinity for stains simultaneously, but two or three of them may often remain stained as before, while the remaining granules of the same nucleus may have entirely lost their stain. At this period in the nuclear degeneration we find small masses of these unstaining, yellowish granules in the cytoplasm, each mass still preserving the form of a nucleus. Later these individual granules wander apart, or those of several nuclei may partially fuse together to produce a larger mass; these larger masses of granules are always enveloped by a clear zone of cytoplasm, sometimes of considerable extent, so that they appear to be situated in vacuoles of the cytoplasm. The degeneration stages of these nuclei are most frequent in the cytoplasm, before yolk balls begin to arise in it; as the latter appear, the remnants of the degenerated nuclei gradually vanish, so that when the cell is filled with the yolk balls all vestiges of these nuclei have vanished. We must suppose that they become assimilated by, or dissolved in, the cytoplasm. These formations, the katabolic changes of degenerating nuclei, can in no way be confounded with stages of yolk development, since the small size, yellowish color, and refrangibility of these granular masses serve to distinguish them sharply from any stage of the yolk balls, even though both are often found in the immediate vicinity of each other.

The nuclei which are destined to become germinal vesicles increase in size to some extent before nucleoli appear in them; they now differ from the connective-tissue nuclei, apart from their greater dimensions, in having a relatively greater amount of chromatin and in being regularly spherical or oval in form. The first nucleoli to arise always lie in close contact with the inner surface of the nuclear membrane (Figs. 214, 216, 219, 220, 224, 225). They usually appear in the form of a thin disc-shaped mass on the inner surface of the membrane, but there is considerable irregularity in the form of this mass, which may be angular or nearly spherical in outline. At the com-

mencement of this *first nucleolar stage* the nucleolar substance appears at only one point in the periphery of the nucleus, and always in the shape of an irregular mass.

*Second nucleolar stage.* — This period is characterized by the formation of other nucleolar masses at various points in the periphery of the nucleus, the successive detachment of all of these from their connection with the nuclear membrane, and their migration towards the center of the nucleus. The commencement of this process is to be seen in very young nuclei, where but a single peripheral nucleolar mass is present ; from the inner side of this mass small particles become divided off (Figs. 219, 224, 225), then each of these particles assumes a more or less spherical shape and wanders to the center of the nucleus ; this process continues until the whole mass of nucleolar substance has reached the center in the form of separate particles (Figs. 217, 218, 223, 227). The peripheral nucleolar mass usually stains less intensely than the portions which have already reached the center of the nucleus. While the first-formed peripheral nucleolar mass is thus gradually wandering to the center, other masses are successively forming at the periphery of the nucleus, and their detached portions successively passing to the center. When a considerable number of these nucleoli have reached the center of the nucleus they naturally come into mutual contact, and then a process of fusion sets in, which results in the coalescence of neighboring groups of nucleoli, so that a smaller number of larger ones are formed. Sometimes this fusion may proceed to such an extent that one single, enormous nucleolus results (Fig. 226), but usually several large nucleoli are the result, these being unequal in size. The irregularity both in the dimensions and the forms of the nucleoli is particularly characteristic for this stage ; thus the individual nucleoli often have elongated processes and angles, and this irregularity is frequently so excessive that the nucleoli within the nucleus appear like smears of ink upon a page (Figs. 226, 227, 230). I think that this irregularity in form may be explained by the assumption that at this stage the substance of the nucleoli is viscid in its consistency, while in the following one, where the spherical form is the rule, its nature must

be more freely fluid. Further, at this period we usually find vacuoles within some of the nucleoli of each germinal vesicle (Figs. 217, 218, 226, 229–231); sometimes no vacuoles are present in any of the nucleoli of a nucleus, but it is the rule that at least one of them, and that usually the largest, contains one or several vacuoles. Sometimes four or five of the nucleoli, which may be very unequal in size, may each have vacuoles. Occasionally a nucleolus contains only one vacuole, and in the latter there may be one or several small solid bodies, which stain like the ground substance of the nucleolus, and may be termed nucleololi; one of the latter may be fused with the inner surface of the nucleolar ground substance (Figs. 217, 218, 230, 231). These nucleololi vary in number and size, and are absent in the greater number of the vacuoles; so no particular significance should be attached to them, since they are probably nothing more than portions of the ground substance of the nucleolus which have become detached from the surrounding substance and have come to lie within the vacuole. During this period the nuclear membrane is thinner than at any other stage, and the nucleus is very noticeably amoeboid in form, the amoeboid processes being much more pronounced than in any of the other nemerteans examined; these processes in reality represent changes in the form of the nucleus, and are not artefacts, since they are seen equally well after preservation in the most diverse fixing fluids (Figs. 226, 227, 230, 232, 233). The nuclear membrane is always particularly thin around these nuclear processes, but, as far as I could make out, never becomes broken.

*Third nucleolar stage.* — The large nucleoli which were present at the end of the preceding stage now commence to fragment into smaller nucleoli, which are more or less equal in size, and then the latter wander towards the periphery of the nucleus; at the conclusion of this period, which must take place in a very short time, since I found only a few germinal vesicles exhibiting it, there are a large number of rather small nucleoli close to the nuclear membrane (Fig. 234). At this time the nucleoli attain their maximum staining intensity; the nucleus usually shows no traces of an amoeboid form, and its membrane has increased in thickness. None of the nucleoli

contain vacuoles ; and in every respect the nucleolar changes during this stage are the very reverse of the preceding.

*Fourth nucleolar stage.* — This is characterized by the gradual degeneration and disappearance of the nuclei (Fig. 235). Small vacuoles arise in them, and these increase numerically, while at the same time the nucleolar substance stains less intensely. Fusion of neighboring nucleoli is very frequent at this time, or perhaps a little time before the nucleoli lose their staining power ; accordingly, in the largest germinal vesicles it is the rule to find a small number of large nucleoli. The nucleoli are not evenly distributed along the periphery of the nucleus, and are often flattened against the nuclear membrane. This nucleolar stage is found only in the largest ovarial eggs, where the nucleus is perfectly regular in outline, without amoeboid processes, and its membrane has attained its greatest thickness.

Since this species is a protandric hermaphrodite, in which male and female sexual products ripen successively in each gonad, I found it at first difficult to determine whether a young nucleus in a given gonad corresponded to a male or to a female cell. But after comparing briefly the spermatogenesis of the other metanemerteans mentioned in this paper, and finding in them that no nucleus in any stage of spermatogenesis was larger than any of the smallest germinal vesicles here figured, I concluded that also in *Stichostemma* no male nuclei can attain the dimensions of even the smallest nuclei of our second nucleolar stage, and hence that all these nuclei were correctly concluded to be germinal vesicles, and not nuclei of spermato-genetic stages.

We notice in the succession of the nucleolar stages described the rhythmic sequence in regard to (1) the position of the nucleoli, (2) their states of fusion and division, and (3) the absence and presence of vacuoles in them ; these successive changes may be expressed as follows :

| | Nucleoli. | | |
|---|---|---|---|
| *Stage.* | *Position.* | *Vacuoles.* | *Fusion, division.* |
| First | peripheral | absent | fusion ? |
| Second | central | present | division, then fusion. |
| Third | peripheral | absent | division. |
| Fourth | peripheral | present | fusion, then division. |

There is without doubt in this genus, as in the other metanemerteans, an extranuclear origin of the nucleolar substance. This is proved (1) by the absence of nucleoli in the nuclei from which the germinal vesicles are derived; (2) by the nucleoli first appearing close to the nuclear membrane. And since yolk globules do not arise in the cytoplasm until nearly the close of the second nucleolar period, when most of the nucleoli are near the center of the nucleus, to the yolk substance cannot be attributed a nucleolar derivation, and other reasons, such as the fact that the yolk balls usually appear at some distance from the nucleus, would contradict such an assumption. The nucleolar substance is apparently formed from an unstaining fluid constituent of the cytoplasm, which after it is taken into the nucleus undergoes a chemical change, since it stains there and is deposited in the form of nucleoli. In the second nucleolar stage, when the formation of nucleoli is at its height, the nuclear sap stains more deeply than at any other period (Figs. 224–227, 233), so that it is probable that at this time nucleolar substance is finely distributed throughout the nuclear sap, as well as in the form of nucleoli. (This staining of the nuclear sap is especially well seen on material fixed with Flemming's fluid and stained with alum carmine.)

In the third and fourth nucleolar stages a few yolk globules are often found in a number of germinal vesicles (Figs. 234 and 235, *Yk. Gl.*); these have probably been taken up by the nucleus from the cytoplasm.

*Chromatin.* — In the nuclei of the first stage, the chromatin is always demonstrable in the form of coarse granules (Figs. 214, 216, 219). In the beginning of the second it may usually be found in the form of a reticulation (Figs 218, 229, 233), but at the end of this stage it is not demonstrable (Fig. 227). In the third and fourth stages it reappears, but now in the form of fine microsomes (Fig. 235); and at the conclusion of the fourth stage short chromatic filaments begin to arise, similar to those described for *Tetrastemma catenulatum.*

8. *Lineus gesserensis* (O. F. M.).

(Plate 24, Figs. 159–177.)

*Yolk.* — The yolk first arises in the cytoplasm in the form of irregular yolk balls, which are much smaller than in the other nemerteans examined (*Yk. Bl.*, Figs. 159, 160, 177); these increase in number and size, the largest sometimes containing vacuoles. In the largest ovarial ova seen (though I had only immature individuals of this species) yolk balls are no longer present, but in their place smaller yolk globules, which in all probability represent fragments of the earlier balls. The yolk usually makes its first appearance in a zone of the cytoplasm, midway between the nucleus and the cell membrane, which is characterized from the rest by a less dense structure (Fig. 177). The extreme peripheral portion of the cytoplasm retains its density longest, as is also the case in the other species. The cytoplasm of the connective-tissue cells (Fig. 159), from which the egg cells take their origin, stains very faintly, while that of the young egg is dense and stains deeply.

*Nucleoli.* — Only three worms out of eighteen sectioned contained ovogenetic stages, and since in these individuals only the earlier stages of this development were found, I am able to describe only the younger stages of nucleolar formation. The egg cell of this heteronemertean contains a single nucleolus ; apparent exceptions will be considered later.

In the smallest nuclei (Fig. 159) of the cell syncytium of the gonads no nucleoli are to be seen ; we find nucleoli for the first time in cells whose nuclei are a little larger and whose cytoplasm commences to stain more intensely. These are the earliest stages of the ovocytes.

Now in these youngest germinal vesicles (Figs. 159, 161, 164, 166) the nucleolus is very frequently peripheral in position, close to the inner surface of the nuclear membrane ; while in the later stages (certain mitotic stages excluded) it is almost invariably never in contact with the nuclear membrane. Further, yolk balls first appear in the cytoplasm when the nucleus contains a nucleolus. These facts, being considered together with the fact that nucleoli are absent in the nuclei of the con-

nective-tissue cells, lead to the conclusion that the nucleolus first appears in the young germinal vesicle, and more particularly, that the substance of the nucleolus is extranuclear in origin, and stands in a genetic relation to the substance of the young yolk balls. The substance of both is homogeneous and stains identically; by fixation in Hermann's fluid, followed by the triple stain of Flemming, the nucleolus and the yolk balls stain a brownish yellow (Fig. 160); by fixation in corrosive sublimate and staining in haematoxylin and eosin both structures are colored a yellowish red (Fig. 177). Still more conclusive is the following observation: while the greater number of the yolk balls may lie at some distance from the nucleus, one or several are very frequently in close contact with the outer surface of the latter, and yolk balls may even be found which are halfway through the nuclear membrane, or which have completely transversed it and lie within the nucleus (Fig. 160). Thus the nucleolus would seem to owe its origin to the substance of yolk balls which have been taken into the nucleus. The very marked increase in the size of the nucleus and the nucleolus is probably caused by a continued process of yolk-ball assimilation on the part of the nucleus. This may be observed in numerous cases where small globules of yolk-ball substance lie within the nucleus, some at its periphery or close to the nuclear membrane, others flattened against the nucleolus (Figs. 160 and 177). By the use of the haematoxylin-eosin stain the nucleolar substance usually stains a little more intensely than the substance of the yolk balls (Fig. 177); this would show that this substance, after being taken up by the nucleus, undergoes a chemical change within the latter. Those yolk balls which are not assimilated by the nucleus remain in the cytoplasm and give rise to the yolk globules, as has been described. Thus the nucleolus probably has an extranuclear origin and represents a portion of the yolk-ball substance taken into the nucleus; its rapid increase in size is due to the addition to it of other similarly assimilated globules of substance.

In the largest germinal vesicles seen (though these were not mature) the nucleolus is usually spherical in form, seldom oval, and homogeneous in structure, except that it sometimes

contains a single large, unstaining globule, which appears as a vacuole (Figs. 162, 175–177); or there may be from one to three minute globules in it, which, when seen in their entirety, present the optical appearance (due perhaps to refraction) of black granules, which might be mistaken for solid bodies. The nucleolus has no limiting membrane. The largest are relatively enormous and stain more intensely with eosin than the smaller ones. There is no clear zone in the nucleus around the nucleolus.

In *Lineus* the study of the metamorphoses of the nucleolus is complicated by the occurrence of nuclei in various mitotic stages. Karyokinetic figures were absent in the ovarial stages of the other nemerteans examined, so that in those species the connective-tissue nuclei and the egg nuclei both stand in the same cell generation, and the germinal vesicle may either be regarded as equivalent to an ovogonium or to a true ovocyte of the first order. In those species no cell generation separates the connective-tissue nucleus and the germinal vesicle, but the latter is merely evolved from the former by a gradual process of differentiation. But in *Lineus* the germinal vesicle is separated from the connective-tissue nucleus by at least one and probably by two or three generations (if the differences in the size of the cells offer a sure criterion). Here, accordingly, the indifferent connective-tissue cell represents an ovogonium, and perhaps another generation of ovogonia may intervene before the germinal vesicle, the ovocyte of the first order, is produced. Of the two individuals on which these nuclear studies were made, I found mitotic stages in only one individual, while none were to be seen in the other individual, though here these nuclei had reached nearly the same degree of development. I have studied the mitosis merely with regard to the behavior of the nucleolus. The most abundant stages were those of the spirem and dispirem, asters and dyasters being much less frequent (Figs. 163, 166, 169, 170–172); the time duration of the latter stages may be less than that of the former. In by far the greater number of the spirem stages one nucleolus was present; it is probably present in each nucleus of this stage, but sometimes may escape observation by being

covered by the chromatic filament or by lying in a part of the nucleus outside of the plane of the section. In this stage, further, two nucleoli are never present; accordingly, in the spirem there is neither a disappearance nor a division of the nucleolus. In the dispirem stage each daughter-nucleus contains one nucleolus (Fig. 171), the two nucleoli being, however, often unequal in size. I found very few aster stages, and these were either so unfavorably placed for study or the chromosomes so densely entangled that I could not determine whether a nucleolus is present in this stage and whether a division of it takes place at this time. The facts determined are (1) that no division of the nucleolus occurs in the typical spirem stage, since here only one nucleolus is present; and (2) that each nucleus of the daughter-spirem has one nucleolus. But I cannot show whether a division of the nucleolus occurs in the time between these two stages or whether the original nucleolus passes over into one of the daughter-nuclei, while in the other one a new nucleolus is produced. In these various mitotic stages the nucleolus usually lies at the periphery of the nucleus, and it is most frequently the case that it is not in contact with the chromatin filament; it preserves its former shape and staining intensity, and apparently does not decrease in size during the mitosis. To be sure, in the karyokinetic stages under consideration it usually appears small in proportion to the size of the particular nucleus, but then it is usually the case in most mitoses, and probably so here, that before the disappearance of the nuclear membrane the volume of the nucleus greatly increases.[1]

Two nucleoli, never quite equal in size, are frequently found in certain small nuclei, which the distribution of the chromatin would show to be in a stage at the commencement of the prophasis of the mitosis or at the conclusion of the metaphasis (Figs. 163, 164–167, 170, 172). As the figures show, all these nuclei which contain two nucleoli are more or less of the same size. Nuclei which are a little smaller than these, as well as those which are larger, invariably contain a single nucleolus.

[1] The chromatin filament has considerable thickness and is apparently a continuous thread; it is looped around the inner surface of the nuclear membrane.

It is probable that the two nucleoli of such nuclei have not arisen by division from a single nucleolus, but are nucleoli which have been developed at different points in the nucleus and which are destined to fuse together later and form a single one. This assumption was based upon the observation of nuclei where two nucleoli lie at opposite poles of a nucleus (Fig. 166) and each is apposed to the nuclear membrane, or where only one occupies such a peripheral position, the other being in the center of the nucleus (Fig. 164). In one figure (Fig. 165) we see a nucleus in which the two nucleoli lie near the center, close together, which might denote the beginning of such a fusion. On a little reflection this explanation of the presence of two nucleoli will appear quite allowable. In the more usual mode of development a larger nucleolus is formed at the periphery of the nucleus, wanders towards its center, and then much smaller masses of nucleolar substance are similarly formed and later fuse with the large nucleolus ; while in the cases under consideration two nucleoli of nearly equal size are produced, either simultaneously or in succession, and these afterwards fuse together. These two nucleoli of nearly equal size cannot be division products of a single primitive nucleolus, since two nucleoli are never found in the larger germinal vesicles.

The nuclear sap of the smaller germinal vesicles does not stain at all ; in the larger ones (Figs. 168, 173–175, 177) it does, and the explanation for this staining may be given by the assumption that there is a dissolution of nucleolar substance throughout the whole nucleus, *i.e.*, of that substance of the assimilated yolk balls which does not enter into the formation of the nucleoli. During the mitotic stages no constituents of the nucleus stain except the nucleolus and the chromatin filament, but these do not stain in the same manner.

At first sight the heteronemertean *Lineus* seems to differ markedly from all the metanemerteans here examined, in that it contains a single, enormous germinal spot. But in *Lineus*, though a single large nucleolus is first formed, it nevertheless grows by the addition to it of much smaller nucleolar globules (*Nut. Gl.*, Figs. 168, 174, 177) which have the same method of

formation and fuse with the former. Were these secondary nucleolar globules in *Lineus* as large as the first-formed nucleolus, and were they all to remain separate from one another, the nucleolar metamorphosis in this genus would correspond to that of the metanemerteans ; accordingly, the difference in the nucleolar production is not very important. (For the nucleolar relations in the other nemerteans, *cf.* my reviews of the papers of v. Kennel, Hubrecht, Coe and Bürger.[1])

### 9. *Siphonophore* (*Rodalia ?*).

(Plate 26, Figs. 204–212.)

(Dr. Conklin kindly loaned me the preparations on which his earlier studies were based ('**91**); these were preserved in alcohol and stained with haematoxylin.)

There were no very young stages of the ovogenesis in this specimen ; I have studied the ova in the egg pouches and in the gonophores, each gonophore containing a single large ovum (as shown by Conklin and Brooks), while in the egg pouches a number of smaller ova may be present.

A single large nucleolus is contained in each germinal vesicle. This is not only large in relation to the size of the nucleus, but is also absolutely probably one of the largest nucleoli ever described in animal cells (Fig. 212). It is always excentric in position, though seldom close to the nuclear membrane. In those younger stages where the nucleus is still near the center of the egg (Fig. 205, and the dorsal cell of Fig. 211)

[1] The only other observations of the yolk development in the nemerteans are those of Bürger ('**90**) on *Drepanophorus*. Near the young germinal vesicle lies in the cytoplasm a homogeneous, deeply staining body, of smaller size than the nucleus, which Bürger assumes may correspond to a yolk nucleus. This body disappears, "und es sammeln sich nämlich, dem Keimbläschen anliegend, in jenem [Plasmahügel] kuglige oder längliche, tröpfchenähnliche Gebilde an, erst spärlich ein einziges, zwei und mehrere, später aber mit dem immer noch fortschreitenden Wachstum des Keimbläschens sich zahlreich vermehrend in grösster Menge. Sie sind durchaus homogen, von mattem Glanze und äusserst tinktionsfähig. . . . Erst nach der Entwicklung des Keimbläschens geht die des Deutoplasmas vor sich und zwar nun auf Kosten der glänzenden Dotterballen, welche aufgebraucht werden und so im reifen Ei verschwinden." In the ripe egg the cytoplasm is granular and stains lightly.

the nucleolus is usually nearer the center of the nucleus than in those more mature stages where the nucleus lies near the periphery of the cell. But in the more mature stages the nucleolus may lie at the animal pole, or the vegetal pole, or at one side of the nucleus, so that no coincidence between the position of the nucleolus and the age of the nucleus can be determined. Thus the nucleolus stands, *e.g.*, in no relation to the animal pole of the more mature nucleus, that pole where amoeboid processes are produced (Figs. 204 and 209). The ground substance of the nucleolus is dense and homogeneous, and stains quite deeply; the nucleoli of the smaller germinal vesicles stain, as a rule, less intensely. In the ground substance of all the nucleoli more or less numerous fluid globules occur, which stain very faintly or not at all, and their presence gives a vacuolated appearance to the nucleolus; those within the same nucleolus are of unequal size, and among them two or three usually occur which far exceed the others in size. Occasionally there is one large central vacuole (Fig. 206), but as a rule the larger ones are peripheral, and may produce prominences of the surface of the otherwise perfectly smooth and spherical nucleolus (Figs. 209 and 212). In one large vacuole (Fig. 212) a finely granular mass was found, though this may have been an artefact. Since in the smaller nucleoli these vacuoles are less numerous and smaller in size, it would seem probable that in stages antecedent to those found by me the nucleolus may be wholly devoid of such vacuoles. The nucleolus has no enveloping membrane, for what at first view appears to be such a structure careful study shows to be merely the result of refraction.

In addition to the single large nucleolus described, there are in the most mature nuclei also from about one to five minute nuclei (Fig. 209). These vary somewhat in size, are perfectly spherical and homogeneous, without vacuoles, and stain more deeply than the larger one. Sometimes they are found in close contact with the nuclear filaments (*cf.* the nucleoli of the second generation in *Tetrastemma catenulatum* and the observations of Rückert ('92) on the germinal vesicles of *Selachii*). These probably have no genetic relation to the large nucleolus, since

they never lie in contact with the latter and are frequently situated at some distance from it. Were they buds from the large one, one would expect to find in them vacuoles such as occur in the large nucleolus, but they never contain vacuoles. In one nucleus (Fig. 207) I saw a disc-shaped mass apposed to the inner surface of the nuclear membrane, which stained more intensely than the chromatin. Such a peripheral mass may be regarded as a substance taken up from the cytoplasm by the nucleus, which, after passing through the nuclear membrane, undergoes a chemical change to such an extent that it stains with haematoxylin. The minute nucleoli may stand in a genetic connection with such a mass of substance, that is, be portions of a substance assimilated by the nucleus and afterwards scattered through the latter. They might serve as nourishment for the chromatin threads with which they are often in contact.

In seven nuclei out of about one hundred or more examined the large nucleoli differed much from the ordinary type described above. In one egg pouch there was a smaller ovum apposed to the animal pole of a larger one (Fig. 211); a normal nucleolus was present in the nucleus of the smaller one. But in the larger ovum two nuclei were present, in close contact with one another, though separated by a membrane (coalesced nuclear membrane). It is in each of these latter nuclei that an abnormal nucleolus is present. Each of these nucleoli is finely granular, without enclosed vacuoles, and stains faintly with haematoxylin ; the one is regular in outline, but the other is jagged at one pole, and a ring-shaped portion of its substance stains more deeply than the remaining portion. In another ovum I also found two nuclei, in each of which was a nucleolus similar to those just described. In still another ovum two nuclei were found in contact with each other, the nucleolus of one of which was similar to those here described, but the nucleolus of the other nucleus was intermediate in structure between these and the ordinary type of nucleoli (Fig. 210). In only one case was such an abnormal nucleolus present within an ovum contained in a gonophore (Fig. 208); in the other six cases the abnormal nucleoli were in ova of egg pouches.

Now what do these lightly staining, granular nucleoli represent? In all except the seven cases here mentioned the nucleolus was always of the deeply staining, vacuolar type, irrespective of its occurrence in ova of egg pouches and of gonophores. The abnormal nucleoli, with one exception, were found in the largest ova of the egg pouches. Types intermediate between the two are represented in Fig. 210. Conklin and Brook's observations, which I can corroborate, show that a number of ova are produced in an egg pouch, but that only one of these passes into a gonophore, and there develops into the ripe ovum, while the others remain behind in the egg pouch and do not reach maturity, but degenerate. I would hold that the abnormal nucleoli described by me are degenerating nucleoli of degenerating ova. All the facts seem to favor such an explanation.

The cytoplasm of the youngest egg cells appears finely granular (it may be an alveolar meshwork). In the largest it was coarsely vacuolar, especially near the center of the cell; I find no evidence of yolk. Conklin and Brooks evidently mistook the vacuoles of the cytoplasm for yolk globules.

No chromatin threads were apparent in the smallest germinal vesicles (Figs. 204–206), but only a fine granulation in the nuclear sap; chromatin threads make their appearance gradually in the larger ova (Figs. 207, 209, 211) and stain more intensely as they increase in number and size. Each thread often has the form of a chain of transversely placed discs; or sometimes it would seem to consist of a large number of short fibrils, placed at right angles to a common longitudinal axis, as is the structure of the chromosomes of the Selachian egg. These threads usually make their first appearance in the neighborhood of the nucleolus, from which they sometimes radiate outwards; only in the largest nuclei are they more generally distributed throughout the nucleus. This fact might show a physiological relation between these two structures. But there is in all probability no genetic connection between the two; rather, the chromatin threads are built up of the minute microsomes found in the nuclear sap of the smaller ova. But the formation of the chromatin threads must be determined by the investigator who has more abundant material at his disposal,

and material which has been more advantageously fixed and stained.[1]

### 10. *Polydora.*

(Plate 28, Figs. 249–281.)

The egg cells of this form, as those of most *Polychaeta*, are derived from the peritoneal cells of the body cavity, the latter cells building pseudoepithelia around the intestine, as well as occurring free in the body cavity. Those in the pseudoepithelia (Fig. 249) are more or less flattened, disc-shaped, while the free cells (Figs. 250–254) are oval in shape, with more regular outlines. Their cytoplasm is not dense, and one or several large vacuoles are frequently found at the periphery of the cell; a delicate cell membrane is present. The cytoplasm of these sexually indifferent cells does not stain with haematoxylin. The nucleus is small, irregular in outline, and contains a few chromatin granules; very frequently the greater part of this substance lies close to the nuclear membrane. I have never found more than one minute nucleolus, and this is almost always close to, or in actual contact with, the nuclear membrane (Figs. 251, 252, 254); in many nuclei I failed to find nucleoli, though in these cases they may have been obscured by the chromatin. I found one division stage of a nucleus (Fig. 249); there were two daughter-nuclei of the same size and form lying close together; the nucleolus of each was somewhat elongate in form (in all others of these cells examined it is spherical), which might show that the nucleoli had been produced by the division of a single one in the mother-nucleus. In many of the smaller free peritoneal cells a peculiar body often occurs (*N. P.* Fig. 253). This is always smaller than the nucleus, more or less spherical, often homogeneous in appearance, and it may stain either deeply red with eosin or faintly with haematoxylin, or in other cases it may not stain at all, but appear as a light yellowish, refractive mass. From the comparative study of a large number of cells containing these bodies it may be determined that they are degenerated nuclei or portions of nuclei. Thus in Fig. 250, which

[1] For other observations on nucleoli of *Siphonophora*, *cf.*, besides the paper by Conklin and Brooks, the review of O. Hertwig ('**78b**).

probably represents the commencement of such a degeneration, there lies close to the nucleus what seems to be a much smaller nucleus, or a portion of one; and I have found all intermediate stages between such a body, which is granular and stains with haematoxylin, and the body reproduced in Fig. 253, which appears nearly homogeneous and stains with eosin. These bodies then seem to be degenerated or cast-off portions of nuclei. We might conclude also that the cells in which these structures are found, are themselves fated not to develop into egg cells, even if they are not degenerating; for no such bodies are to be seen in the cytoplasm of the true egg cells.

These peritoneal cells have the morphological value of ovogonia. Those which are destined to become ova seem to become detached from the pseudoepithelial connection, but in such a way that they do not become detached singly, but portions, each of which is composed of a number of cells, become loosened from the epithelium. Thus the earliest ovogenetic stages are to be found in strings of cells arranged radially around a common longitudinal axis, each such string of cells situated free in the body cavity (Fig. 270 represents a portion of such a string). At the one end of such a cellular string lie, densely grouped, the numerous mitoses of the ovogonic stages, while the remaining portion of the string is usually composed of young ova, *sensu strictiori*. I have never found mitoses in cells which lie singly in the body cavity.

The first change noticeable in the ovogonium leading to the formation of the ovum consists in (1) the increase in the size of it and of its nucleus, and (2) in its cytoplasm gradually staining with haematoxylin. This deep blue staining of the cytoplasm, accompanied by its increasing density and the loss of the vacuoles in it, continues from now on until yolk granules begin to arise in it, when the cytoplasm commences to stain faintly with eosin and loses its dense structure. At the conclusion of the ovogonium rest stage the nucleolus has increased a little in size, accompanying the growth of the nucleus.

The next stage is a mitosis. Whether there is more than one mitotic generation separating the ovogonium from the ovum I have not been able to determine; the slight differences

in the size of the mitoses hardly afford a satisfactory criterion for deciding this point (Figs. 255–261). All the typical stages of the prophase and metaphase are to be found, though only in the arrangement of the chromatin, for I have been unable to find either centrosomes or achromatic spindle. After careful study of a large number of these dividing nuclei I find the nucleolus to persist in the nucleus throughout the mitosis. Further, it appears to retain its original size throughout this process, without any diminution in volume. Thus the nucleolus seems to be retained without change in the spirem and aster stages of the prophasis. In the dyaster stage (Fig. 258) each pole of the nucleus usually contains a nucleolus, so that the nucleus contains two nucleoli; and when the nuclear division is completed, *i.e.*, when in one and the same cell two nuclei occur in close contact with each other, in the aster as well as in the spirem of the metaphasis, each daughter-nucleus has its own nucleolus (Fig. 257). Now the ovogonium contains only one nucleolus, so that we must assume either (1) that a division of the nucleolus has taken place during the mitosis, or (2) that to one of the daughter-nuclei is allotted the whole original nucleolus, while in the other nucleus a new one is produced. I have not seen any dividing nucleoli in these mitoses, their small size being a great obstacle to their study. But I should judge that such a division occurs, for these reasons: (1) the nucleus of one or of both the daughter-nuclei has sometimes a somewhat elongate form (Fig. 257); and (2) in later stages of the ovum proper I have found dividing nucleoli, and these cases would show that if such divisions take place in stages subsequent to the mitosis they might also occur during the mitosis. The two cases of division of the nucleolus found are here figured (Figs. 264 and 265), and in each of the elongate nuclei is a dumbbell-shaped nucleolus lying in the longitudinal axis of the nucleus; in these figures the two halves of each nucleolus appear unequal in dimensions, but this is so because neither of these nucleoli happened to lie wholly in the plane of the section. I have found numerous other cases of elongated nuclei, each with an elongate nucleolus without any median constriction (Fig. 270). These facts would show that a division

of the nucleolus may take place during the mitosis, and probably does so.

After the completion of the mitosis just described, each daughter-nucleus, which now has the value of a germinal vesicle, first passes through the spirem stage of the metaphasis and then enters upon the stage of synapsis, namely, the nucleolus has a more or less central position, and all the chromatin of the nucleus becomes grouped immediately around it (Figs. 264–266, 270, 271, 278), the peripheral part of the nucleus being transversed by only a few fine, unstaining strands of substance (linin ?). All intermediate grades between this and the preceding stage of the nucleus may be found. This is not an artificial appearance caused by the use of a particular preservative, since it is equally demonstrable on preparations fixed with aqueous or alcoholic corrosive sublimate, sublimate with acetic acid, Flemming's fluid, and alcoholic solution of picric acid; only after the use of Perenyi's fluid is this arrangement of the chromatin not found, but this fluid seems to be rather a poor one for most cytological study. It cannot be an artefact, since this appearance is found only in ova of a certain size but not in those which are larger; thus it cannot be produced by the resistance offered by the cell membrane to the penetration of the fixatives, since this membrane is much thicker in the larger ova. This central arrangement of the chromatin then represents a definite stage of the germinal vesicle concomitant with the first appearance of yolk globules in the cytoplasm.[1] So at this point we may briefly describe the yolk development and then return to the changes of the nucleolus.

The yolk first arises in the cell during the stage just described, that is, immediately after the conclusion of the spirem stage of the metaphasis. It appears in the form of small globules (*Yk. Gl.*, Figs. 262–264, 266, 270, 271), most of which are arranged close to the outer surface of the nuclear membrane, the first globules rarely arising at a distance from the nucleus. At this period they stain less deeply than later. The yolk

[1] This stage of synapsis (Moore) appears to be characteristic of the anaphase of the last spermatogonic and spermatocytic division in all the higher animals, and no doubt can any longer be expressed of its representing an artefact.

rapidly increases in amount, spreading from the region of the nucleus (which is central) to the cell periphery. In the largest ovarial ova the cytoplasm is densely filled with larger and smaller yolk globules; the larger ones appear homogeneous when stained with eosin (Fig. 269), but the Ehrlich-Biondi stain shows them to be composite masses of small globules.

The nucleolus rapidly increases in size, at a somewhat greater proportionate rate than the nucleus itself. It is now large enough for its structure to be clearly made out : it consists of a homogeneous ground substance, which seems to stain more deeply with eosin as it grows larger; a limiting membrane is clearly demonstrable in the largest nucleoli (Figs. 271–277, 279–281) after staining by the Ehrlich-Biondi method or after fixation with Flemming's fluid, though it does not differ chemically or in structure from the ground substance and is only a thin layer of the latter in which vacuoles never occur. At the close of the metaphasis of the mitosis small vacuoles make their first appearance in the ground substance of the nucleolus (Figs. 263 and 270). There are only a few of them at the start, but their number rapidly increases as the nucleolus grows larger, until there are large numbers of them in its center (Figs. 268 and 269). They are always more numerous at the center than at the periphery of the nucleolus, and usually first appear at the former point. On preparations stained with eosin the small vacuoles appear either as clear spaces or as black granules, according to the focusing of the microscope; after the use of the Ehrlich-Biondi stain they become a light grayish color (note the contrast, — that in the eggs of *Doto* and *Montagua* the nucleoli appear as black granules only after treatment with the latter stain); after fixation in the fluid of Flemming the substance of these vacuoles is of a lighter color than the ground substance. This vacuolar substance is homogeneous, and is probably of a thin, fluid nature. With the growth of the nucleolus the number of the vacuoles becomes very great, though their size does not seem to increase. In the nucleoli of the largest germinal vesicles examined the vacuoles no longer retain their original spherical form, but become mutually confluent to some degree, not in such a manner as to pro-

duce one or a few large vacuoles, but as to produce an irregular canicular network of vacuolar substance in the nucleolus (Figs. 272–277, 279–281). This process often goes so far that in the largest nucleoli the deeply staining ground substance may appear in the form of a skein of threads, or merely of scattered granules surrounded by vacuolar substance. Especially on preparations stained by the Ehrlich-Biondi method is the skein-like arrangement of the ground substance well marked. I have no doubt that it was the observation of similar nucleoli in like stages which led Carnoy to the assumption of a "nucléole-noyau," that is, a nucleolus with a limiting membrane, and containing a wound thread of chromatin ; it is probable that Carnoy mistook the reticulum of the true ground substance of the nucleolus for chromatin, and considered what is really vacuolar substance to be the original ground substance ; only studies on the genesis of a nucleolus can explain its various components.

In the largest ova found in the body cavity the nucleolus reaches its maximum size (Figs. 279–281). It contains a greater amount of vacuolar than of ground substance, and instead of being regularly oval, as it was before, is often quite irregular in form, and very frequently apposed to the nuclear membrane (a position not noticed in any of the preceding stages). Whether this irregularity of form denotes the commencement of a degeneration of the nucleolus I cannot say, since I had no preparations of the stages of reduction.

Two nucleoli were found in only two germinal vesicles (Figs. 262 and 266), and in a spirem stage of an ovogonium three small nucleoli were present in one nucleus (Fig. 261). In the hundreds of other resting nuclei examined a single nucleolus was invariably present. These exceptional cases must, therefore, be considered abnormal, and not typical for certain stages of the nucleus.

In the larger germinal vesicles there is a peculiar body in contact with the nucleolus, which remains to be described. This body (*nx.*, Figs. 272, 274–277, 279, 281) is homogeneous, somewhat refractive, and lies either in close contact with the surface of the nucleolus, projecting beyond the periphery of the

latter, or (and this is the rule for the largest, irregular nucleoli) it is imbedded in the peripheral portion of the nucleolus ; in the former position it is concavo-convex, in the latter, biconvex in outline, always being thickest in its median diameter. With the Ehrlich-Biondi staining method it almost invariably colors yellowish, and in only one or two cases did it stain somewhat similarly to the ground substance of the nucleolus ; after fixation in Flemming's fluid, and staining with safranin, gentian violet, and orange G., it always appeared yellowish, while the ground substance remained wholly unstained. The largest nucleoli, *i.e.*, those of the largest germinal vesicles, have always at least one of these bodies in contact with their surface, but quite frequently two may be found on opposite sides of the nucleolus, and in one case I found three (Fig. 277). Those of different nucleoli vary slightly in their dimensions, but my observations give no clue as to their origin. All that can be said of their growth is that in the smaller nucleoli they lie upon the surface of the latter, while they are sunk into the peripheral portion of the larger nucleoli. It differs both chemically and structurally from the ground substance of the nucleolus, and from the vacuolar substance ; and it would seem to be derived from some part of the nucleus outside of the nucleolus, since it at first lies upon the surface of the nucleolus. This body may be comparable to the " Nebennucleolus " described by Flemming in the egg of *Anodonta ;* but I have found no structure in any of the other ova here examined which is identical with it.

Yolk globules are assimilated by the nucleus from the cytoplasm, though without the production of amoeboid processes. Such assimilated globules are usually of small size, but sometimes large, compound ones are taken into the nucleus (Figs. 267–269, 272, 274, 280); they occur most frequently singly or in small masses close to the inner surface of the nuclear membrane (Figs. 274 and 280) in almost all of the larger germinal vesicles, and in a few cases some globules may be found near the center of the nucleus. Careful observation shows that the yolk globules really occur within the nucleus, and are not artificially removed there by the knife in sectioning. Usually these

stain in the same manner as those contained in the cytoplasm. But occasionally from one to three of the larger globules (Fig. 267) in the nucleus stain much more intensely than the others, though intermediate degrees of staining are to be found between these largest, most deeply colored ones and the smaller, less deeply stained ones; so that there can be no doubt of the genetic relation of the two kinds. By staining with eosin these largest yolk globules in the nucleus stain almost or quite as deeply as the nucleolus itself, so that at first I mistook them for nucleoli; but that they are chemically metamorphosed yolk globules and not nucleoli is shown, even leaving aside the fact that all intermediate forms may be found between them and the less deeply staining globules of the cytoplasm, by the fact that vacuoles are never found within them. By the Ehrlich-Biondi staining method no color differentiation was to be obtained for the larger and smaller yolk globules of the nucleus. But nevertheless I would think that these large yolk globules (or accumulations of such globules) which have been taken into the nucleus from the cytoplasm and there have undergone some degree of chemical change, possibly stand in genetic connection with that body which is apposed to the nucleolus in the larger germinal vesicles, and which has been described in the preceding paragraph.

*Chromatin.* — We found the chromatin in the primitive peritoneal cells and in the youngest ovogonia to be arranged in the form of granules (Figs. 250–254). In the following mitoses it is arranged in the form of a spirem, then of chromosomes, and again of a spirem (Figs. 255–261). Just after the conclusion of the spirem stage (of the metaphasis) it comes to lie in a more or less dense mass around the nucleolus, this mass appearing to be composed of a reticulum of short fibers (Figs. 263–266, 270, 271, 278). In all these stages the chromatin is marked by its deep blue staining with haematoxylin. After the last stage described it gradually departs from the close vicinity of the nucleolus and becomes evenly distributed throughout the nucleus. But when it has thus become diffused it does not stain with haematoxylin as before, but appears in the form of a very large number of minute microsomes, which

appear not to stain at all, and of a few delicate fibers, which stain a lilac color (Figs. 267–269). As the germinal vesicle increases in size these chromatin fibers gradually become thicker and more numerous, commence to stain more deeply with haematoxylin, and gradually connect together to build a chromatin reticulum ; the minute, unstained microsomes still occur between these fibers. Finally, in the largest nuclei at my command, and ones which had been fixed with the fluid of Flemming and stained by the triple stain of this cytologist, we find, in addition to the abundant unstained microsomes, short, rod-like masses of chromatin, which stain deeply with gentian violet, and each appears to be formed of a row of granules or thickened discs (Fig. 280). Whether the minute microsomes are true chromatin or are lanthanin (oedematin) granules is open to question ; the latter assumption might be the correct one. We notice two remarkable phenomena in the chromatin changes just depicted : (1) the grouping of the chromatin in the center of the nucleus, around the nucleolus, at the completion of the mitotic stages ; and (2) immediately subsequent to the preceding, the lilac stain of the chromatin after haematoxylin. Now, concomitant with the former of these two phenomena, the yolk makes its first appearance in the cytoplasm, and as we have shown above, usually in the close vicinity of the nucleus. It would be quite erroneous to conclude that the yolk globules are in any way produced by the chromatin, as *e.g.*, by a migration of chromatin particles out of the nucleus ; for in this stage all the chromatin is removed from the periphery of the nucleus. On the other hand, however, I would suggest the hypothesis that the reason for the chromatin being removed from the periphery of the nucleus is because at this period the peripheral portion of the latter is chiefly concerned in the assimilation of yolk substance from the cytoplasm. In support of this assumption the fact may be recalled that in the following stage the chromatin fibers are stained a lilac color, as if they were stained with eosin, as well as haematoxylin, and not as before, simply with the former stain ; this would show that during this period there is an addition of a cytoplasmic substance to the chromatin fibers, perhaps allied to the substance of the yolk globules, and

this substance would superinduce the lilac staining of the chromatin threads. This addition of a probably nutritive substance would seem necessary, in order that the amount of the chromatin continue to increase as the nucleus itself grows larger. Subsequently all that nutritive substance attached to the chromatin threads would seem to become metamorphosed into chromatin, since in the largest germinal vesicles these threads again stain a deep blue. And as a matter of fact, the quantity of the chromatin must increase with the growth of the ovum, since it can easily be demonstrated that in the larger nuclei there is an absolutely greater amount of this chromatin present than in the nuclei of the primitive peritoneal cells.[1]

11. *Piscicola rapax* (Verr.) (= *Pontobdella rapax* Verr., which Dr. Percy J. Moore assures me is a true *Piscicola*).

(Plate 29, Figs. 300–316.)

(The ovary is a tubular, contorted sack; from its inner surface numerous smaller, likewise tubular (round on cross-section), acini project into its cavity, each acinus containing numerous ovogenetic stages, the least mature of which lie at its proximal end, the most mature at its distal. These several acini are not continued as far as the external opening of the ovary, but their distal ends apparently open into a large ovarial cavity, and the ova drop into this cavity before they can arrive at the external genital opening. Each single acinus of this leech may be compared to either of the two whole ovaries of *Ascaris*.)

The youngest ovarial stages are small ovogonia in stages of mitotic division (Fig. 300). In them no nucleoli were to be seen; a minute nucleolus might be present in each of these nuclei and be obscured by the dense mass of chromatin. In all stages subsequent to these a single nucleolus is present in the nucleus (now a germinal vesicle) until the pole spindle is formed; in the smaller nuclei the nucleolus is usually oval, in the larger ones spherical. The growth of the nucleolus keeps

[1] For the researches of other authors on germinal spots of polychaetous annelids, *cf.* the reviews of the papers of Korschelt ('89, '95), Graff ('88), Giard ('81), Vejdovský ('82), Eisig ('87), Fraipont ('87), Mead ('95), Fauvel ('97), Michel ('96), and Carnoy ('84).

pace proportionally to that of the nucleus (Figs. 301–304). Then vacuoles arise in the nucleolus, these being neither very numerous nor very minute (Figs. 304–310, 312–316). The time when these vacuoles first arise is very variable, though in the majority of cases they do not appear before the nuclear sap begins to stain red. The size of the nucleolus does not always stand in the same proportion to that of the nucleus. Its ground substance is dense, stains deeply with eosin, and no limiting membrane is present; but by the use of the double stain Lyons blue and acid carmine, whereby the nucleolus stains blue and the chromatin red, a deep red line appears to surround the nucleolus: I cannot determine whether this line is a nucleolar membrane or a layer of chromatin, or whether it is merely an appearance caused by the refraction of the nucleolus.

When the nucleolus first appears it is usually situated at that pole of the nucleus opposite the chromatin mass and is not in contact with the nuclear membrane (Fig. 301). In nuclei of intermediate size, before the nuclear sap commences to stain with eosin, it is most frequently in contact with the nuclear membrane (Figs. 302–304); but in the largest germinal vesicles it is never in contact with this membrane, though often lying excentrically in the nucleus.

As soon as the germinal vesicle has nearly, or quite, attained its maximum dimensions (quite frequently, however, in those of still smaller size) two very noticeable changes take place in it: (1) the chromatin assumes a different form and stains differently (these chromatin changes shall be delineated later); and (2) the nuclear sap, which had heretofore remained colorless or was merely of a light lilac shade (by the double stain haematoxylin and eosin), now becomes a yellowish-red color, so that the nuclei in this stage may be easily distinguished from those of preceding ones (Figs. 304, 305, 307–310, 316). Simultaneously two changes occur in the nucleolus: (1) it stains no longer a deep red with eosin, as before, but a yellowish red, and appears more refractive; and (2) the several vacuoles within it gradually fuse together and so produce a larger one, which has usually a central position. The fluid, structureless

substance of the vacuole stains more faintly than the ground substance of the nucleolus, and has much the same color shade as the nuclear sap. In certain germinal vesicles, which appear to be of a somewhat later stage of development, numerous small globules (*n.D.*, Figs. 306 and 310) are scattered through the nuclear sap; they stain with eosin a little more deeply than the last-named nuclear portion, vary in number and size, and have no regular distribution. In one case (Fig. 316), which stood in a stage immediately antecedent to the pole spindle formation, where there was a centrosome at either end of the nucleus in the cytoplasm (the nuclear membrane had not yet disappeared), such globules were present in the nucleus; so that we may infer that these globules are one of the latest formations in the germinal vesicle before the pole spindle is formed. I have not found any stages between the stage just described and the perfectly formed spindle (Fig. 311). About fifty or sixty ova were examined in the stage of the first pole spindle, and in all of them the nucleolus had completely disappeared, and no trace of it could be found either in the nucleus or in the cytoplasm.

What has been the manner of this disappearance of the nucleolus? Its total disappearance must occur within a relatively short time, since otherwise one would expect to find stages showing this process. The observations which I was able to make would demonstrate at least the mode of the commencement of the vanishing of the nucleolus. We have seen that when the germinal vesicle has attained its greatest size or, in some cases, a little before its maximum size is reached, its nuclear sap stains red; therefore some substance must be suspended in the caryolymph at this period which was not contained in it before. Now such a substance must have been derived either from other elements of the nucleus or from the cytoplasm. It has probably not been derived from the cytoplasm, since the nuclear membrane at this stage has its maximum thickness and hence could not be easily penetrable; and also there is no appearance of any similar substance in the cytoplasm, since no yolk globules or other nutritive elements seem to be present, but the whole cytoplasm (at least the

nodules of its meshes) stains a lilac-blue color. And since it is wholly improbable that it should be derived from the chromatin we must conclude that it takes its origin from the nucleolus. In other words, a substance emanates from the nucleolus and dissolves in the nuclear sap, and this process must be regarded as the commencement of the dissolution of the nucleolus. In support of this conclusion is the fact that in many germinal vesicles the nuclear sap stains most intensely in the neighborhood of the nucleolus (Fig. 309). Further, the minute red-staining globules which later occur in the nuclear sap must also be nucleolar in point of formation, *i.e.*, be either a substance given off in globular form from the nucleolus, or be accumulations (perhaps chemically changed by the action of the nuclear sap) of that nucleolar substance which has already diffused through the nucleus. Of importance in this connection is the fact that these globules are often found in contact with the nucleolus (Figs. 306 and 316). In all preceding stages the nucleolus is regularly oval or spherical in outline, but in the largest germinal vesicles not only may the size of its contained vacuole be increased to such an extent that the original ground substance forms only a thin shell around it (Figs. 308, 312, 314), but also its outline becomes frequently irregular (Fig. 313); and in one case I found it broken at one pole, so that its large vacuole communicated with the cavity of the nucleus (Fig. 315). A morphological change in the shape of the nucleolus which seems to take place with great regularity consists in the indentation of the nucleolar wall at that point where it is thinnest (Figs. 308, 314, 316). It would seem that the pressure from without, *i.e.*, the pressure of the nuclear sap, being greater than the pressure of the fluid within the vacuole, would cause the nucleolar wall to be pressed in at that point where it is thinnest. The fact remains that the nucleolus persists in the nucleus until a very short time before the production of the pole spindle, and when the latter is formed no trace of it can longer be found in any part of the nucleus or cell. And since there is no reason for supposing that it is extruded from the cell we must assume that it dissolves within it. The red-stained substance and small globules

within the nucleus would show that dissolution commences in the nucleus; and we must assume that when the nuclear membrane has disappeared the cytoplasmic substances which then come into contact with the nucleolus would cause its rapid and total dissolution. It may be remarked that in the region of the fully formed spindle (Fig. 311) no trace of the red-stained nuclear sap is longer to be seen; accordingly this sap with its contained nucleolar substance must either have been distributed through the cytoplasm or have been chemically changed by that portion of the latter which immediately surrounds the spindle.

In the ovary no ova are to be found which have advanced beyond the production of the first pole body, so that the formation of the second pole body must occur after the egg has been discharged from the ovary; I had no material at hand to enable me to determine the relation of the nucleolar substance in the female pronucleus.

Of considerable morphological interest are the metamorphoses of the chromatin in the various ovarial stages. In those small ovogonic mitoses (Fig. 300) from which the true egg cells (first ovocytes) are derived aster and dyaster stages are to be found; with the lens used for this study (the homogeneous immersion $\frac{1}{12}$ of Zeiss) I could not determine the form of the chromosomes. As the ovum increases in size the dense chromatin mass of the aster gradually loosens, until up to the time when the nuclear sap commences to stain red (Figs. 301–304) the chromatin is arranged in the form of rather numerous granules, which are situated mostly close to the nuclear membrane. Thus far the chromatin has stained intensely blue, with the double stain haematoxylin and eosin; but when the nuclear sap begins to stain with eosin a marked change takes place in the character and arrangement of the chromatin; it now stains a lilac color, often more reddish than bluish, and has no longer a peripheral position, but becomes arranged in the form of threads, sometimes in the form of a small number of loops, the two ends of each loop being joined together (Figs. 304, 305, 307, 309). In some of the larger germinal vesicles absolutely no trace of chromatin can be found (Fig. 316). In the equator

of the first pole spindle (Fig. 311) lie twelve small chromosomes, which stain an intense blue black with haematoxylin and have an oval or slightly elongate form. It remains for investigators working with more abundant material and with stronger microscopical lenses, to penetrate more deeply into these phenomena of the chromatin changes, but it would seem that the chromosomes of the first pole spindle have the value of either tetrads or dyads. The lilac or even reddish stain of the chromatin at a particular period would seem at first sight to be due to the assimilation by the chromatin of that nucleolar substance diffused in the nuclear sap ; but even as probably it might be due to the mere penetration of this substance between the individual microsomes of each chromatin thread, without any chemical change of the chromatin substance (Fig. 309). The red-staining globules in the nuclear sap, which I have assumed to be of nucleolar derivation, cannot be considered as metamorphosed portions of chromatin substance, since they vary so considerably in size and number ; this point needs to be emphasized, since in some of the larger germinal vesicles no trace of chromatin is to be seen, and it might be thought by some one that these globules, which occur in such nuclei, represented the supposedly absent chromatin. (Platner, '89c, had, in *Aulastomum* seen only nucleolar fragments and overlooked the true chromosomes.) Where in the largest germinal vesicles, before the formation of the pole spindle, the chromatin appears to be absent in the nucleus, we must assume that it is merely obscured by the large amount of diffused nucleolar substance.

In the first pole spindle (Fig. 311), after treatment with Flemming's fluid or with corrosive sublimate, the mantle fibers have a remarkable thickness and appear even thicker than in Fig. 311 ; they stain a reddish-lilac color with the haematoxylin and eosin stain, not a lilac blue, as do the rays of the asters and the cytoplasm ; I could not determine whether they extend quite to the centrosomes. I am also unable to decide whether each chromosome lies upon a single spindle fiber which extends from centrosome to centrosome, or whether its ends are connected with separate fibers. The centrosomes are rather large,

refractive granules, and stain with eosin ; they were present in one egg, close to, and opposite, the two poles of the nucleus, before the nuclear membrane had disappeared (Fig. 316), so that they may be extranuclear in origin. The radiations of the asters are very clear, especially after fixation in Flemming's fluid, and may be traced nearly to the cell membrane. Immediately around each centrosome a central portion of the aster is differentiated, namely, an attraction sphere (in the terminology of van Beneden), and this differs from the remaining portion in staining less intensely, and appears to be quite sharply bounded from it. In this attraction-sphere the cytoplasmic granules are smaller and more densely grouped, so that at first sight it might appear to consist of a homogeneous "archoplasm," but careful study shows that in it the cytoplasmic microsomes are arranged in radial rows around the centrosome, and each of these rows appears to be continuous with a ray of the outer aster. Or, to express it differently, the microsomic rays of the sphere extend to the centrosome, but this terminal part of each ray differs from the remaining distal portion in that its microsomes are smaller and closer together. Thus in *Piscicola* the finer structure of the attraction-sphere seems to have much resemblance to that of *Ascaris*, as described by Kostanecki and Siedlecki (*Arch. mikr. Anat.*, 48, 1896).

It remains to describe the mode of arrangement of the ova within each ovarial acinus. The proximal, small end of the latter is filled with small ovogonia (the youngest stages), and from mutual contact these are polygonal in form (Fig. 300). As we proceed towards the distal end of the acinus (Fig. 301) the ova not only become gradually larger, but have a different arrangement, in such a manner that they become epithelially grouped along the wall of the acinus, each cell having a pyramidal shape, with its apical end directed towards the central cavity of the acinus. A little more distally in the acinus (Figs. 302 and 303), the ova become not only larger, but fewer of them are to be found on a given transverse section of the acinus ; the individual ova have more of an oval shape and become separated from one another. Now when we proceed still further towards the distal end of the acinus (Fig. 304) we find a single

ovum commencing to outstrip the others in point of size, *i.e.*, in rapidity of growth, until we reach a point where this fortunate cell nearly fills the whole cavity of the acinus, driving the neighboring ova aside. Those cells which come into contact with such a rapidly growing ovum, as well as those in more proximal portions of the acinus which did not chance to lie close to the wall of the acinus, do not develop further, but disintegrate, and various stages of such disintegration may be seen in the cavity of the acinus, such as irregular cells with a nucleus, those which have lost their nuclei, and finally refractive cytoplasmic masses which stain deeply with eosin (the cytoplasm of the developing ova stains with haematoxylin). Perhaps such degenerated masses of cellular substance are destined to be assimilated by their more fortunate brethren. Often a number of such degenerating ova are to be seen grouped at one pole of a large ovum, and these cases present a certain similarity to cleavage stages, the large ovum resembling a macromere, the others micromeres. It is not difficult to find an explanation for the disintegration of certain of the ova, for only those close to the wall of the acinus can procure nourishment in amount sufficient for their growth, since this nourishment must be derived through the wall of the acinus from the body cavity (there being no yolk in the ova); and the peripherally situated ova must obtain all the nourishment thus furnished, so that those in the center of the acinus simply die for want of food. Further, a particular ovum of those placed peripherally, if it procures a greater amount of nourishment than its neighbors do, because, *e.g.*, of being in contact with a greater surface of the wall of the acinus, grows faster than the others and, pushing them aside, eventually gets full control of the whole amount of nourishment, so that a slight advantage at the start would increase in value in a geometrical ratio. Here, accordingly, we have a beautiful example of that process termed by Roux "der Kampf der Theile ums Dasein," that cell becoming a mature ovum which has succeeded in obtaining the greatest amount of nourishment. It is also interesting to note the position of the nucleus within the growing ovum; in all the younger stages of the egg it is placed in that part of the cell

which is nearest to the wall of the acinus, *i.e.*, nearest to the source of the food supply ; only then does it come to occupy a central position within the cell, when the latter has attained its maximum size and the thickness of the cell membrane shows that the cell is assimilating little or no nourishment from without.[1]

### *b. Somatic Cells.*

#### 12. *Ganglion Cells of Doto.*

(Plate 21, Figs. 36–49.)

(I have studied those nerve cells which occur in the cerebral, pleural, and pedal ganglia. Three kinds of these cells may be readily distinguished and described in succession : (1) colossal cells, which are found only in the cerebral ganglion; (2) cells of medium size; and (3) small cells.)

*Colossal ganglion cells* (Figs. 43–49).—The number of the nucleoli in the nuclei of these cells varies from about six to thirteen; they are also variable in regard to the position which they occupy in the nucleus, and though always excentrically placed they never lie in contact with the nuclear membrane. Sometimes all the nucleoli in a given nucleus are of approximately equal size, but as frequently one or two are several times larger than any of the others. Where such larger nucleoli occur along with a number of smaller ones, the former are usually vacuolar in structure ; sometimes nearly all the nucleoli contain vacuoles, in other cases none of them are vacuolar. Quite often the nucleoli in a nucleus show slight differences in their staining intensity, and one of them may stain quite differently from the rest (Figs. 44 and 46). None of the nucleoli have limiting membranes. No cases of nucleolar division were found, unless those cases where two nucleoli lie near to one another may represent the completion of such a division.

*Ganglion cells of medium size* (Figs. 37–42).—In these the nucleoli vary in number from one to four, two or three being the rule. Those of the same nucleus frequently show differences in size and form, as well as slight staining differences. In only one

[1] For the observations of other writers on germinal spots in *Hirudinea*, *cf.* O. Hertwig ('**76**), Leydig ('**49**), Whitman ('**78**), and Platner ('**89c**).

case (Fig. 41) I found three nucleoli of approximately equal dimensions and homogeneous ; usually they vary somewhat in size and contain vacuoles. The shape of the nucleoli is either spherical or oval, or it may be irregular; certain ones stain scarcely at all, and appear granular : these might represent cases of degeneration.

*Smallest ganglion cells* (Fig. 36). — Here a single nucleolus is the rule, though two may occasionally be found. They are spherical or oval, and vary considerably in size. Vacuoles do not seem to occur in them, though they might well escape observation from the small dimensions of the nucleoli, which often renders it difficult to distinguish the nucleoli from the larger chromatin granules.

In all these ganglion cells the chromatin appears in the form of small granules, but on a preparation fixed with Hermann's fluid and stained with Lyons blue (Fig. 45) it appeared as a network ; in this preparation the granules seemed to be united by fine fibers, which stained less intensely than the granules. But even here the connecting threads might consist rather of linin than of chromatin, since the solution of Lyons blue employed by me stained all the nuclear substances except the nuclear sap (paralinin). Such fibers often appear to radiate outwards from the surface of the nucleoli, as if the latter were suspended by them. The nucleoli always stain differently from the chromatin.

There is, as a rule, a relatively small amount of nucleolar substance in the cells of the second and third types in comparison with most of the other nuclei which I have examined ; but the nuclei of those of the first type, on the contrary, usually contain a relatively large amount of this substance, for not only may one or two of the nucleoli in a nucleus be quite large, but also a considerable number of nucleoli are frequently present.

### 13. *Ganglion Cells of Montagua pilata* (Verr.).

(Plate 22, Figs. 90–97.)

(The same types of cells may be roughly distinguished as in *Doto*.)

*Colossal ganglion cells* (Figs. 90–92, 94–97).—In the nuclei of these there are never more than from one to three nucleoli, which neither contain vacuoles nor become noticeably irregular in size, as is the case in *Doto*. Most frequently only a single nucleolus is present. It is the rule that they are oval and not spherical, though in some cases they may appear perfectly spherical; perhaps the great majority of them are oval and seem to be spherical only when they do not chance to be longitudinally sectioned. Their substance is perfectly homogeneous, without a limiting membrane. When two or three occur in the same nucleus they are usually of approximately equal dimensions (Figs. 94 and 95). Further, it would seem to be the rule that when one nucleolus is present in a nucleus it is larger than any one of the two or three which may be found in other nuclei; but, nevertheless, the relative amount of nucleolar substance seems to vary in different nuclei.

*Ganglion cells of medium size* (Fig. 93). — Here one or two nucleoli are present in each nucleus, and these are of homogeneous appearance.

*Smallest ganglion cells.*— The nucleoli are similar to those of the corresponding cells of *Doto*.

On a preparation preserved in Flemming's fluid I find many of the nucleoli present a different structure from those fixed with corrosive sublimate or Kleinenberg's fluid. Thus many of them do not appear homogeneous, but finely granular and refractive (Figs. 96 and 97). On the surface of such nucleoli occur small, refractive, yellowish globules, which appear black or yellow, according to the focus of the microscope; some of them are very small. These never occur within the nucleolus, but only on its periphery. They may easily be distinguished from the chromatin granules by their rounded form and high degree of refrangibility, as well as by their deeper yellow color (this preparation had been stained with haematoxylin and eosin, but the nuclei had not become stained, probably owing to too long a fixation in the fluid of Flemming). Numerous other nuclei on the same sections showed none of these globules, and none were to be seen on preparations which had been differently preserved. Accordingly, I consider them to be artefacts,

caused by (1) the direct action of the fluid of Flemming, or more probably (2) they might be post-mortem exudations of the nucleoli, which might well be produced before the slowly penetrating fixative had reached to the cells in question. At any rate, they cannot be regarded as normal structures. Do they represent the "Kernkörperchenkreis" of Eimer?

The chromatin, as in *Doto*, occurs in the form of granules, which are connected by fine fibers. After fixation with Kleinenberg's fluid a clear space encloses each nucleolus (Figs. 93 and 94); but this space is not to be found after fixation in other fluids.

As in *Doto*, the nuclei of the colossal ganglion cells contain a relatively greater amount of nucleolar substance than do those of the second and third types. But in the former genus there are in the colossal cells from about six to thirteen nucleoli, and these vary noticeably in size and structure, while in *Montagua* there are only from one to three, which are always homogeneous and usually quite equal in dimensions. Why should there be this marked difference in the form and number of the nucleoli?[1]

### 14. *Ganglion Cells of Piscicola rapax* (Verr.).

(Plate 23, Figs. 134–136.)

In the ganglia of the brain occur cells of different dimensions. Each nucleus contains most usually a single small spherical nucleolus; seldom are there two present, and in these cases they are unequal in size. None of the nucleoli contain vacuoles. They are excentric in position, but are never in contact with the nuclear membrane. These nucleoli are small in proportion to the size of the nucleus.

### 15. *Muscle Cells of Lineus gesserensis* (O. F. M.).

(Plate 21, Figs. 51–56.)

(The nuclei of the circular muscular layer of the body wall were studied. Those of *Cerebratulus lacteus* Verr. are essen-

[1] For other observations on nucleoli in ganglion cells of molluscs, *cf.* the reviews of the papers of Pflücke ('95), Leydig ('83), and Rohde ('96).

tially similar to those of *Lineus;* in the metanemerteans they are too small for satisfactory study.)

These nuclei are very variable in shape, all extremes being found between ovoid or oval and elongate rod-like forms. But they are rarely angular. I have remarked in a previous contribution that the nuclei of the muscle cells are more variable in form than those of the cells of any other tissue in the nemerteans, and now I would offer the following explanation for this variability: when the muscle fiber (a single, smooth fiber with attached nucleus constitutes a muscle cell) contracts, this contraction must produce likewise a contraction (shortening) of the nucleus; but when the fiber expands the form of the nucleus must become more elongate, corresponding to the elastic extension of the fiber, for the fiber cannot contract without causing a shortening of its nucleus, since the latter is closely adherent to it.

One very small nucleolus is usually to be seen in each nucleus (Figs. 51–54, 56); sometimes it does not appear to be present (Fig. 55), but whether in these cases it is absent or only escapes observation by reason of its minute size, it is difficult to decide; in the greater number of nuclei it may be seen by careful focussing of the microscope. It most usually lies very close to the center of the mass of nucleoplasm, so that if the nucleus be larger at one pole than at the other it is situated in the larger end, while in elongate nuclei, of nearly equal diameter throughout, it usually lies at an early equal distance from both ends of the nucleus. The nucleolus may be said, as a general rule, to occupy the center of the nuclear substance, and is not often markedly excentric; in none of the other cells examined in the course of these investigations did the nucleoli show a similar tendency to occupy the center of the nucleus. The nucleolus always stains differently from the chromatin.

The relative amount of chromatin varies in different nuclei. It is always found, after the action of various fixatives, to occur in the form of small granules, which are connected by delicate irregular fibers, which stain exactly as the granules do. The nuclear sap stains faintly with haematoxylin (this has not been shown in the figures). The nucleolus is either in contact

with chromatin granules or with fibers of chromatin, which pass between it and the nuclear membrane; there is never a clear space around the nucleolus, but it seems to be held in position by the chromatin.

### 16. *Muscle Cells of Piscicola rapax* (Verr.).

(Plate 29, Figs. 325–337.)

(The nuclei of the longitudinal muscle layer of the body wall were studied. For the examination of the different stages of these nuclei worms of different sizes must be studied; I examined the nuclei of leeches of about 6 mm. in length, where the cells and their nuclei are smallest, as well as of larger and fully mature individuals, where these cells and their nuclei attain their maximum dimensions.)

In the smallest nuclei (Fig. 325) a single nucleolus is invariably present and lies centrally; it is of medium size, more or less oval in outline, and contains a varying number of small vacuoles. In larger nuclei it becomes larger and more elongate in form, lying in the longitudinal axis of the nucleus (Figs. 328 and 331); at the end of this stage its greatest dimensions are reached. Next commences a process of fragmentation of this original nucleolus into a number of smaller nucleoli, which are of different sizes. There appears to be little uniformity in the mode of this nucleolar division (Figs. 327, 329, 332, 333): the nucleolus may become dumbbell shaped and then divide into two larger pieces; or when much elongated it usually breaks simultaneously into a number of consecutive portions; or buds of nucleolar substance may be divided off from its surface. This segmentation is not strictly dependent upon the size of the nucleus, nor upon the size or form of the nucleolus. The fragmentation continues, the larger daughter-nucleoli also dividing, until in the largest nuclei (those of the mature worm) as many as twelve small nucleoli may be present, which are irregularly distributed through the nucleus (Figs. 335–337). In all these stages at least some of the nucleoli contain vacuoles, though they have not been reproduced in all the corresponding figures.

All the nucleoli of the largest nuclei are thus produced by a

series of divisions from the single original one. This division usually commences, then, when the form of the nucleus changes from the original oval to a more elongate shape. It seems probable that this elongation of the nucleus may directly cause the division of the nucleolus, since the long axis of the latter coincides with that of the nucleus; and were the nucleolus in any way fixed in position in the nucleus, the nuclear elongation would draw out the nucleolus and cause it to break into fragments. But the division of the daughter-nucleoli does not always take place in the direction of the long axis of the nucleus, so that some other factor might be at work to produce this division.

The chromatin is arranged in the form of a reticulation (Fig. 326). The nuclei of the younger cells are usually regular in outline, but those of the larger ones become very irregular; this irregularity of the contours of the nuclei is more marked by fixation with corrosive sublimate than with Flemming's fluid, so that it might be regarded as an artefact caused, *e.g.*, by the obstacle offered to the rapid penetration of the preserving fluid by the dense outer (fibrillar) layer of the cytoplasm in the largest muscle cells.

### 17. *Blood Corpuscles of Doto.*

(Plate 22, Figs. 98–101; Plate 23, Fig. 102.)

(These cells are usually to be found abundantly in the cavity of the cirratida and of the sheaths of the tentacles, though their number varies greatly in different cirratida. They lie in the meshes of the loose network of mesenchym cells, either singly or grouped together into bundles. I have been unable to find them in other parts of the body. These cells appear to be free mesenchym cells, with perhaps the function of blood corpuscles.)

There is always a single large nucleolus, which is usually very large in proportion to the size of the nucleus. It varies in form from a perfect sphere to an elongate oval. The nucleolar substance is usually homogeneous, but in some cases it is granular (Figs. 99–102) and then it stains faintly as if it were

undergoing a degeneration. Quite frequently a small spherical granule lies in the center of the nucleolus and this always stains more intensely than the surrounding substance (Figs. 99–102). In only about half a dozen cases, out of hundreds of cells examined, did I find attached to the surface of the nucleolus one or two much smaller bodies, which also stained less intensely (Figs. 100 and 101). Can it be that in certain cases the nucleolus becomes differentiated into a "Hauptnucleolus" and a "Nebennucleolus," in which case these small bodies would represent the "Nebennucleoli"? In certain of the cirratida of a young individual the nucleoli of the greater number of nuclei were situated at that pole of the nucleus directed towards the median axis of the cirratidum, *i.e.*, those in the nuclei on the right side of the cirratidum were in the left-hand poles of the nuclei, and those in the nuclei of the left side of the cirratidum (as seen on sections) were placed at the right-hand poles of the nuclei. I did not observe this regular position of the nucleoli in the cirratida of the other individuals sectioned and hence would conclude that it was not a normal phenomenon, but an osmotic consequent of the fixing reagent.[1] The size of the nucleolus preserves approximately the same ratio to that of the nucleus.

The nucleus is either spherical or oval in outline. The apparent arrangement of the chromatin varies according to the fixative employed. After picro-nitro-osmic acid (Fig. 102) it appears granular; after Hermann's fluid (Figs. 99–101), in the form of delicate fibers which radiate from the nucleolus to the nuclear membrane; after alcoholic solution of corrosive sublimate (Fig. 98) we find a few fibers radiating from the surface of the nucleolus, but the greater amount of the chromatin appears in the form of granular masses, which lie mainly near

[1] In a previous paper ('**96**) I figured for the nuclei of those mesenchym cells which surround the distal end of the ventral nerve cords of *Cerebratulus lacteus*, the nuclei with their chromatic masses pressed against that side of the nuclear membrane which was situated nearest to the central point of the section. At that time, I regarded this excentric position of the chromatin as a normal but peculiar phenomenon; but now, on comparison with the cells of *Doto*, I am convinced that it is an artefact produced by the osmotic action of the fixing reagent.

the periphery of the cell, so that the nucleolus is surrounded by a clear space. These nuclei thus offer a suggestive object lesson, to teach how careful one must be in the determination of the form of delicate cellular structures by the study of preserved material.

Cells which are isolated have a spherical form; those grouped together are polygonal, owing to their mutual pressure (Figs. 99 and 101). A cell membrane is present. The cytoplasm is for the most part finely granular; portions of it, however, are always more dense and stain more deeply than the former portion; there are great individual differences in different cells (Figs. 100 and 102). Often the cytoplasm is more or less vacuolar or a clear space may partially surround the nucleus and a similar space be present between the cytoplasm and the cell membrane, this space being transversed by a few radiating fibers. Such spaces are best shown after the action of the fluid of Hermann; they are seldom to be seen after fixation in picro-nitro-osmic acid; but whether a coarse alveolar layer of cytoplasm at the periphery of the cell be normal or be an artefact, there are certainly marked differences in the structure of the cytoplasm in neighboring cells, and these differences might be regarded as the morphological changes corresponding to functional phases in the cells. Cases of degenerating cells are numerous, and may be recognized by their faint staining properties and by their granular appearance.

### 18. *Giant Cells of Doto.*

(Plate 30.)

(These enormous cells, which are the largest cells in the body, not excepting the ova, lie at the anterior part of the body just behind the head region and are closely apposed to the folds of the nidamental gland. They do not produce a closed mantle on the outer surface of this gland, but either are isolated or occur in small groups of from two to four cells each. In each individual their number appears to be about thirty or forty. These cells do not seem to have any open communication with the neighboring tissues, and I cannot

conclude from their structure what their function is; perhaps they have a function similar to that of lymph glands. Such cells are absent in *Montagua*.)

The form of these is a more or less polyhedral one, caused by the pressure of the surrounding organs (Fig. 339). The nucleus is relatively and absolutely very large and is very variable in form, sometimes irregularly oval, sometimes with obtuse or pointed processes, or again concavo-convex, that side being convex which lies near the nuclear membrane (on a transverse section such a nucleus appears sickle shaped). The chromatin is arranged in the form of rather coarse granules (Figs. 339 and 342), which after fixation in Hermann's fluid (Fig. 340) appear to be the nodal points of a reticulum.

The nuclei (Figs. 338–346) are numerous, vary in number from about six to about forty, and are irregular in size. Their shape is usually oval, seldom perfectly spherical, though quite frequently, as the figures show, they may be more or less elongate or even very irregular in form. Vacuoles are frequently present in them. The nucleoli stain as do all true nucleoli, but different degrees of staining density may be observed in the nucleoli of the same nucleus (Figs. 338, 342, 346). In two cases, one of which is here figured (Fig. 342), a dense ring of chromatin was found around a nucleolus, but such cases, judging from their infrequency, must be regarded as very abnormal, if not attributable to the action of the fixing fluid. Divisions of the more elongate nucleoli certainly take place. Thus I have observed dumbbell-shaped nucleoli in three cases (Figs. 343, 345, 346), and Fig. 340 probably represents a stage just after a division, where two smaller nucleoli have apparently been divided off from a larger one, one end of the latter being drawn out to a point. Thus it might seem that the large number of nucleoli are produced by divisions of a smaller number of larger nucleoli. The variability in the size, form, and number of these nucleoli recalls those of the subcuticular gland cells of *Piscicola* (*cf. infra*); but in these cells of *Doto* I have been unable to make out different morphological phases.

The cytoplasm of these cells is also remarkably differentiated (Fig. 339). In a given cell certain portions of the cytoplasm

may be dense and stain deeply; other portions are less dense in structure, with a corresponding less intensity of stain; and still other portions of the cell substance appear structureless and do not stain at all. The cytoplasm in at least a portion of the peripheral area of the cell is always dense and deeply staining; rarely is the cytoplasm in the whole cell of this dense structure. With low powers of magnification (*e.g.*, Zeiss Obj. C, oc. 2 or 4) there may appear to be either several cavities in the cytoplasm or a single large one at one side of the nucleus. These differentiations of the cytoplasm (which fixation in corrosive sublimate or in Hermann's fluid bring out always in the same manner) probably denote certain metabolic states of the cytoplasm, but it would be difficult to determine from the structure alone to what physiological processes these states might correspond. There is no definite secretion produced by the cytoplasm, *i.e.*, no secretion with a definite form. As has been noted, a wholly or nearly wholly clear space often occurs in the cytoplasm at one side of the nucleus; such a space usually lies at that margin of the nucleus situated closest to the center of the cell, and the nucleus may often surround it to some extent. Where the nucleus comes into contact with this space its membrane is thinnest and its outline irregular, and quite frequently this margin of the nucleus is produced into long, irregular, amoeboid processes, which extend into the space in question and pass around it. These appearances would show that the nucleus stands in a certain functional relation to the metabolic changes of the cytoplasm, not improbably that it assimilates certain substances produced in the latter.

To return to the nucleoli, I cannot find any genetic connection between these structures and the cytoplasm. They are usually grouped near the center of the nucleus, and though often quite peripheral in position, never come into contact with the nuclear membrane, nor are they found in the amoeboid processes of the nucleus. It will be necessary to study very young individuals of this mollusc in order to determine the mode of nucleolar development.

The cell (Fig. 339) is developed by a delicate membrane, which seems to be interrupted at no point on the surface of the

cell. The cell has thus no external openings and no ducts or fibers which penetrate into the enveloping tissues.

### 19. *Subcutical Gland Cells of Piscicola rapax* (Verr.).

(Plate 25; Plate 26, Figs. 198–203.)

(These cells lie for the most part in the body cavity, between the body muscular wall and the intestine. Two modifications of them may be distinguished: (1) those at the ends of the body, near the suckers and in the wall of the latter, which are comparatively small, and the relatively short cell ducts of which open at all points of the surface of the body at the ends of the body and on the inner surface of the suckers; these seem to resemble the second modification in all respects except size; (2) the larger type of these gland cells, which I have studied exclusively, are massed together in that portion of the body cavity which extends from the region a little anterior to the brain, nearly to the posterior end of the body, the greater number of them being in contact with the inner surface of the body wall.)

In order to find all the functional stages of these cells one must study preparations of worms of various dimensions, since all the stages cannot be found in a single individual; I made consecutive series of sections of seven different individuals, the smallest being about 6 mm. in length, and the largest being fully matured. The remarkable cycles of the nuclear and cell stages, to be described below, were equally well discernible with all three of the fixatives employed, namely, Flemming's fluid and alcoholic and aqueous solutions of corrosive sublimate; various double stains were used.

These cells, when they reach their fullest dimensions, are so enormous that they may be readily seen with the naked eye. Their single ducts all open on the surface of the body, between the epithelial cells, a little anterior to the region of the sexual pore; their openings are at this point equally numerous on the dorsal, lateral, and ventral sides of the worm. The most posterior gland cells of the body send their ducts a distance of four-fifths the total length of the body before they open on the

surface of the latter, these ducts transversing a large number of body segments (in certain of the enchytraeid *Oligochaeta* there have been described subcutical gland cells whose ducts pass through a number of segments, but I believe that they are not of the same relative length as those of *Piscicola*). Each cell has its own duct, the latter being morphologically merely a process of the cell (Figs. 178, 181, 202); and as these individual ducts run in bundles parallel to one another, on their way to the surface of the body, they become closely apposed to one another, but there are apparently no open communications between the several ducts, nor do they unite to form larger, compound ducts. The ducts of those gland cells which are situated behind the sexual pore necessarily have an anterior direction, while those which are situated near to the head end of the animal send their ducts posteriorly. The duct departs from the cell more or less at right angles from its distal end, *i.e.*, that end which is usually directed towards the central axis of the worm. Since the greater number of these cells become filled with secretion only when the worm is sexually mature, and since they all open on the surface of the body near the sexual pore, they have probably the same function as the clitellar glands of the *Oligochaeta;* after these observations had been completed I found that Bourne ('84) had described such gland cells in *Pontobdella* as "clitellar glands," but he made no observations on their finer structure.

In studying the cycle of the structural changes of these cells two main morphological periods may be distinguished: (1) the *prophasis*, from the immature cell to the cell filled with secretion; and (2) the *metaphasis*, from the time when the cell begins to discharge its secretion until it becomes re-formed into a functionally immature cell again. I have no means of determining whether a given cell becomes filled with secretion only once a year (as, *e.g.*, at the period of sexual maturity) or whether it may secrete several times in succession during the sexual period. At any rate, all appearances lead me to conclude that it secretes periodically, most probably once during each period of sexual maturity. I have found no evidences that it secretes only once and then dies to become absorbed by the other tissues of the body; in other words, there were no

evidences of cell degeneration or of a formation of new cells, so that we must conclude that each of these cells continues to functionate periodically during the whole time of the existence of the leech.

We may now describe in succession the prophase and the metaphase of the structural changes.

*Prophasis* (Figs. 178–196). — In the smallest cells found in the youngest leech examined no trace of secretion is present (Figs. 178–180). In these the nucleus is usually central in position, with a delicate chromatin network, and with a single, most frequently oval, nucleolus, in which one or a few small vacuoles are commonly present. Around the nucleus, and filling the cell duct, is a somewhat dense cytoplasm, which becomes more vacuolar at the periphery of the cell. The chief cytoplasmic changes from now on are as follows (I have not figured these changes, since they may be briefly characterized): that portion of the cytoplasm close to the nucleus gradually becomes more dense and begins to stain differently from the rest, and then becomes quite homogeneous; most frequently there is a layer of this homogeneous substance between the nucleus and the cell duct, only that portion of the cytoplasm at the proximal end of the cell, as well as a thin layer around the homogeneous substance, retaining its primitive appearance. Next, this homogeneous mass gradually breaks up into the numerous secretion corpuscles (Fig. 181, *Secr.*), the shape of the latter being ovoid after fixation in corrosive sublimate, but more spherical after the action of Flemming's fluid. These secretion corpuscles stain at first just like the homogeneous substance, but gradually commence to stain otherwise, and in the functionally mature cell stain differently from the primitive cytoplasm, as well as from the homogeneous substance from which they were derived. The whole cell thus gradually becomes filled with these small corpuscles, until finally no trace of the original cytoplasm is to be seen, except a few faintly staining fibers. The cytoplasm which fills the duct undergoes the same morphological changes as that of the cell body just described, so that the first secretion corpuscles in it are the derivatives of its own substance; the cytoplasm of the

duct and of the distal portion of the cell are as a rule the first portions to become differentiated into the secretion. At the end of the prophase the cell has attained its maximum size, and the duct its greatest diameter, both containing hundreds of the mature secretion corpuscles lying in an unstained, structureless fluid. The duct in all stages is always larger at its proximal than at the distal end, though it narrows very gradually.

But the most interesting morphological changes are those of the nucleus. While the secretion is being produced in the cytoplasm the nucleus increases rapidly in size, and at the same time becomes very irregular in form, until in the nearly physiologically mature cell it attains enormous dimensions and sends out through the substance of the cell long branching processes, which anastomose with one another and some of which reach even to the cell membrane (Figs. 178–196). Korschelt has described ('89) branched nuclei in the spinning glands of certain insect larvae, which are somewhat similar to the nuclei here delineated. The nucleus attains its greatest dimensions and its most marked degree of ramification when there is the greatest amount of the homogeneous substance in the cell, *i.e.*, just before this substance becomes metamorphosed into the secretion corpuscles. At this stage we find the greater portion of the nucleus situated at the proximal part of the cell, and from that point it sends out irregular branches which envelop the mass of homogeneous substance, and which penetrate into it. At this period, further, no two nuclei are alike in form, so that it would be in vain to attempt to figure all the shapes which they may assume. The nuclear membrane becomes very thin, often scarcely perceptible, around the branched processes. I know of no other nuclei which are more interesting in point of size and variability of form than these ; and it would well repay accurate investigation in the endeavor to decide in what way they may influence or modify the cytoplasmic changes which are simultaneously taking place, for they obviously have a close physiological connection with the formation of the cellular secretion. Since the nucleus undergoes a rapid process of growth in these stages, we are obliged to assume that it is taking up substances from the cell body ; but

it probably does not assimilate the mature secretion corpuscles, since when the latter are produced, as we shall see, the nucleus commences to retract in size and to withdraw its processes. As the nucleus increases in size its chromatin reticulum becomes looser, as if it were elastically stretched by the expansion in volume of the nucleus; the chromatin is continued into the ramifying processes of the nucleus.

The nucleolar changes during the prophase are as follows: in the immature cell there is invariably a single rather large nucleolus, which occupies a more or less central position in the nucleus (Figs. 178–181, 184); it may be either oval or spindle shaped, and most frequently contains one or several small vacuoles. Its substance appears homogeneous after treatment with corrosive sublimate, granular after the action of the fluid of Flemming, and has no limiting membrane; in all its stages within the nucleus it stains very intensely, though always differently from the chromatin. Now as the nucleus increases in volume so also does the nucleolus, though at first at a relatively more rapid rate than does the former; and in growing larger it gradually becomes more elongated, rod shaped, and at this stage is most frequently in contact with the nuclear membrane (Fig. 182). When it has taken up this peripheral position its period of most rapid growth commences, so that at this stage there is a proportionately greater amount of nucleolar substance in the nucleus than at any other period in its history. When it is apposed to the nuclear membrane it has at first more or less the form of a rod (often of a slightly curved rod), but as its substance commences to increase in volume this rod shape gradually becomes changed and the nucleolus becomes bent inwards (towards the center of the nucleus), frequently in the form of a V, an S, or a W, though there is marked variability in regard to the form it may assume, since no two nucleoli can be found at this stage which have exactly the same form (Fig. 189). It is about this time that the nucleolus attains its greatest staining density. Then this large and irregularly shaped nucleolus leaves the nuclear membrane and begins to fragment into pieces, which are very irregular in shape and variable in number and size; the nucleolus may show thereby

a number of constrictions, or buds of nucleolar substance may project from its surface ; it may first break into two larger pieces, and then these may fragment further, or it may at once break into a number of pieces which are irregular in their dimensions (Figs. 185–188, 190, 191, 193). These fragments gradually wander apart from one another, the nucleus now being larger and already somewhat irregular in shape ; and at the same time each of the primitive nucleolar fragments divides into smaller pieces of unequal size, until when the nucleus has attained its greatest dimensions and most pronounced degree of ramification it contains a very large number of irregular nucleoli, which are unequal in their dimensions (Figs. 194–196). The figures given of this last stage show only sections of nuclei, and since as many as five or six sections may be made of one of these colossal nuclei (my sections were between 3 and 5$\mu$ in thickness), not one of these figures shows more than a portion of the total number of nucleoli in these largest nuclei ; in some of the latter nuclei I compute the number of the nucleolar fragments to be at least three hundred. But the total mass of nucleolar substance in these largest nuclei is certainly considerably greater than the mass of the primitive nucleolus at the time of its greatest size ; accordingly, though the division products of the primitive nucleolus might constitute the greater part of the nucleolar substance in the largest nuclei, they do not constitute all of it. Therefore there must be a formation of new nucleolar substance after the primitive nucleolus has divided, *i.e.*, a production of nucleolar substance not derived from the primitive nucleolus ; I cannot determine the manner of formation of this new nucleolar substance, but would suggest that either new nucleoli are formed, or that the fragments of the primitive nucleolus increase in size by the addition of new nucleolar substance to them. The greater number of nucleoli in the largest nuclei are collected in or near the thicker portion of the nucleus and few or none lie in the branched processes ; they are at this time seldom in contact with the nuclear membrane. Only a few of them contain vacuoles, and those which do may be regarded as derivatives of the primitive nucleolus, the vacuoles of the latter still being preserved in its daughter-nucleoli.

A nucleolar change now occurs which I have never seen paralleled, and to my knowledge no similar morphological change has ever been described. At the time when the homogeneous substance of the cell is commencing to differentiate into the secretion corpuscles, the nucleus begins to withdraw its branched processes and to decrease in size; while so doing it discharges its nucleoli into the cell body (Figs. 197–199). There can be no doubt of the genuineness of this process, since I have examined at least two hundred nuclei at this stage, which showed all intermediate stages between nuclei which had discharged only a few nucleoli and those which had discharged all except a single one of their nucleoli into the cell. The study of these nuclei gives the impression that successive contractions of the nucleus take place, whereby at first all the more peripheral nucleoli, and later those which are more central in position, become successively extruded, for in the cell two or three more or less parallel rows of nucleoli may be found, or more properly speaking, concentric circles of nucleoli (Figs. 197 and 198). In some cases I have observed nucleoli which were halfway through the nuclear membrane, but by far the greater number of the nucleoli are found either within or without the nucleus, and this would prove that the contractions of the nucleus are sudden in their action. I think that it is the sudden contractions of the nucleus which alone cause the expulsion of the nucleoli, for as the nucleus diminishes in volume its chromatin network may be seen gradually to become closer and denser, and the pressure within the nucleus becoming greater than the pressure without it, the nucleoli, not being fixed in position, are forced out into the cell body where there is comparatively little pressure, since the secretion corpuscles are not densely grouped, but lie scattered through a thin and structureless fluid substance. The nucleoli, when they have arrived in the cell body, are not found in equal number at all points around the nucleus; accordingly they are probably not discharged from all sides of the nucleolus in equal number, but only there where the nuclear membrane is thinnest (it is probably thinnest at those points whither the nuclear processes had withdrawn themselves). But though the nuclear membrane appears to be thinner at some

points than at others, there are no visible pores in it, so that the nucleolar substance must be squeezed through the nuclear membrane itself. When one takes a sponge filled with water and presses it in the hand the water is forced out of it in the form of jets or columns, which are radial to the surface of the sponge; exactly similar seems to be the method of the discharge of the nucleoli in the case at issue, except that the nucleus is itself actively contracting. Thus we find the greater number of the nucleoli which lie in the cell body close to the surface of the nucleus to be irregularly columnar or rod like in shape (Fig. 198) and radially grouped around the nucleus. Those which lie nearer the periphery of the cell, however, and which had probably been discharged by a previous contraction of the nucleus, are more irregular in form, and their axes have a less regular position with regard to that of the nucleus. Further, those ends of the rod-like nucleoli in the cell which are directed towards the surface of the nucleus are usually more attenuated than the opposite ends, *i.e.*, a nucleolus lying in the cell close to the nucleus has often the form of a pyramid the apex of which is directed towards the surface of the nucleus, and this form we would expect to result in the squeezing of a more or less viscid substance, like that of the nucleoli, through the nuclear membrane. I give only two figures showing the stage of the discharge of the nucleoli from the nucleus, simply in order to save time in the drawing of the numerous nucleoli; but my preparations show very clearly all the stages of this process: one has only to examine sections of the mature leech to find them in abundance. The extrusion of the nucleoli continues until only about twenty, then a dozen, then four or five, and finally only a single nucleolus (Fig. 199) remains in the nucleus; corresponding to these successive states of the discharge of the nucleoli we find cells in which only a few nucleoli, and then those in which the greater number of the nucleoli, lie in the cell body. One nucleolus always remains in the nucleus, though this one appears to differ in no wise from those which are discharged. Those nucleoli which lie in the cell body (Figs. 197–199) differ from those in the nucleus in their lesser density, greater size, and different reactions to certain stains (we shall

return to the chemical change later); in other words, the substance of those nucleoli which have come to be situated in the cell body undergoes a physical and perhaps a chemical change in this portion of the cell, and their expansion in volume might be accounted for on the ground of there being a smaller degree of pressure in the cell body than there is in the nucleus.

It will be noticed that the prophase and the metaphase of the cell body and of the nucleus do not exactly coincide in point of time, the metaphase of the nucleus commencing earlier than that of the cell body. Thus the nucleus attains its greatest dimensions and most diverse ramification at the time when the cell body contains the greatest amount of homogeneous substance, and the nucleus enters on its metaphase (diminution in volume, retraction of processes, expulsion of nucleoli) when the secretion corpuscles are only commencing to arise in the cell body. At the beginning of the metaphase of the cell body (when the latter is filled with the secretion corpuscles and commences to excrete them) the nucleus has already assumed a nearly spherical or oval form, has greatly decreased in size, and has discharged most of its nucleoli into the cell, *i.e.*, the nucleus has advanced already some distance in the path of the metaphasis.

The metaphase of the cell body (Figs. 198–203) commences when the cell is filled with the secretion corpuscles, all traces of the previous homogeneous substance being absent, and begins to discharge them through its duct. During this process the cell gradually decreases in size, and the primitive cytoplasm again comes into view, at first in the form of delicate fibers. When the cell has shrunk to about one-third of its former size (the diameter of the duct does not decrease quite so rapidly, since it may be still full of secretion corpuscles after they have all disappeared from the cell body) the nucleus has simultaneously decreased in size, but with greater proportionate rapidity than the cell body, and so at the close of the metaphase (Fig. 202) the nucleus reaches its smallest relative size. The latter contains at this stage invariably a single nucleolus, of spherical or oval form, very regular in outline, and exactly similar to the nucleolus at the commencement of the anaphase except that

it does not appear to contain vacuoles. The nucleus itself is somewhat elongate and irregular in outline, and, owing to its maximum degree of contraction (a characteristic of the end of the metaphase), its chromatin builds a dense network within it. A study of the cell body at this stage allows us to follow the morphological changes undergone by those nucleoli which had been discharged by the nucleus (Figs. 198–203). The cytoplasm gradually assumes a reticulate or a somewhat granular structure, and finally a most regular vacuolar or alveolar structure. As the cell body decreases in size the discharged nucleoli lying in it gradually stain less deeply, they lose their rod-like form, and no longer remain isolated, but all the nucleolar substance in the cytoplasm gradually becomes confluent, and becomes arranged in the form of a coarse, irregular network of substance distributed in the cytoplasm, and readily distinguishable from the latter by its different staining properties (Figs. 201–203). By a hasty inspection this network of nucleolar substance might appear to represent branches of the nucleus, but a careful study shows that at this period of its growth the nucleus has no branches. As the cell continues to become smaller the amount of nucleolar substance in the cytoplasm gradually becomes less and less, first the network at the periphery of the cell disappearing, then that in the vicinity of the nucleus, until at the conclusion of the metaphase no nucleolar substance is any longer to be seen in the cytoplasm. I am unable to determine whether it is finally discharged through the cell membrane or whether it becomes metamorphosed into cytoplasm ; it certainly is not excreted through the cell duct, since no nuclear substance occurs in the latter, and at this stage the duct is no longer an open tube, but all the secretion corpuscles having been expelled from it, it is again filled with cytoplasm. The suggestion may be made that at least a portion of this nucleolar substance remains in the cytoplasm, so that in the succeeding prophase the nucleolus within the nucleus might find the material necessary for its growth in the nucleolar substance suspended in the cytoplasm ; thus there might be, in the history of the nucleolar substance, periods of its expedition into the cytoplasm alternating with those when it is again taken

into the nucleus. And that in the prophase the single nucleolus of the nucleus derives the material necessary for its further growth from the cell substance, seems highly probable when we recall the fact that at the time of its most rapid growth it is usually apposed to the nuclear membrane, which would denote that it is taking up a substance which penetrates that membrane from the side of the cell body.

We have alluded to certain chemical changes which occur in the nucleolar substance when discharged from the nucleus during the metaphase of the latter. These staining differentiations and the coloration of the cytoplasm as observed on five different preparations are as follows (the first preparation was fixed with Flemming's fluid, the others with corrosive sublimate).

*First preparation* (Ehrlich's haematoxylin, two hours ; eosin, ten minutes): cytoplasm pale lilac; nucleoli in the nucleus, and when first discharged from it, reddish or rusty brown; nucleolar substance at the end of the metaphase lighter in color.

*Second preparation* (gentian violet in aqueous solution, twenty-five minutes ; eosin, four and one-half minutes) : cytoplasm very faintly stained ; nucleoli in the nucleus deep violet, those in the cytoplasm yellowish red.

*Third preparation* (Ehrlich's haematoxylin, one hour ; eosin, five minutes) : cytoplasm pale pink ; nucleoli in the nucleus, and when first discharged from it, purple ; nucleolar substance in the cytoplasm at the end of the metaphase pure blue.

*Fourth preparation* (Ehrlich's haematoxylin, forty minutes ; eosin, five minutes) : nucleolar substance within and without the nucleus yellowish red ; cytoplasm of a paler red.

*Fifth preparation* (Mayer's acid carmine, twenty minutes ; Lyons blue, five minutes): cytoplasm unstained ; nucleoli in the nucleus, and, when first discharged, bluish green; nucleolar substance at the end of the metaphase reddish purple in the cytoplasm. These methods of double staining show that the nucleolar substance, when discharged from the nucleus, undergoes some chemical change in the cytoplasm ; and they serve to distinguish, further, this substance from the true cytoplasm.

I have no material of *Piscicola* after the breeding season, and accordingly could not follow the changes of these gland cells in their metamorphosis from the end of the metaphase to the commencement of the prophase. But these two end stages do not differ much from one another, since the cell at the former stage differs from that of the latter merely in that its nucleus is smaller and more irregular in shape.

It is not difficult to determine the sequence of the stages described ; only in the smallest individuals do all the stages of the prophase occur, and only in the largest those of the metaphase.

### 20. *Mesenchym Cells of Cerebratulus lacteus* (Verr.).

(Plate 29, Figs. 315a, 316a–324.)

(I have described these cells in a previous contribution ('96), and so shall treat of them in this place mainly with regard to their nucleoli.)

The smallest nuclei (Figs. 316a and 317) are densely filled with chromatin, and nucleoli appear to be absent ; the nuclear sap also stains with haematoxylin, so that these nuclei may be easily recognized by their deep stain and sometimes nearly homogeneous appearance. I have made a careful examination for nucleoli on preparations stained by the Ehrlich-Biondi method, as well as with haematoxylin and eosin, and am certain that nucleoli are either wholly absent or, if present, must be very minute in point of size. Such, then, is the structure of the smallest nuclei, namely, those found in the body cavity, and those of the smallest cells of the pseudoepithelia lining the body cavity.

The non-continuous pseudoepithelia of the body cavity are layers of differentiated mesenchym cells, which differ from the primitive cells in their greater dimensions and more oval or spherical outlines (the undifferentiated cells are bipolar or multipolar, with long branching processes). In these larger cells we find for the first time a spherical, deeply staining nucleolus. Now the size of the latter stands in a pretty constant ratio to that of the nucleus. Further, in the smallest

nuclei which contain nucleoli, from one to three of the latter occur, and one or all of these are frequently found in close contact with the nuclear membrane (Figs. 315a, 318, 320), while in the largest nuclei observed only a single nucleolus is present, and this one is relatively large and is always at or near the center of the nucleus, never at its periphery (Figs. 319, 322, 323). In connection with the problem of the origin of this nucleolus we recall those small granules contained in the cytoplasm, which I have ('96) termed nutritive particles. These particles (*Nut. Gl.*) stain with eosin quite as intensely as the nucleolus, and in the smallest cells are either wholly absent or present in only small number; but in the larger cells they are usually much more abundant, or when not more numerous they are of greater size, and are often quite densely grouped around the nucleus. It would seem probable that the nucleolar substance is derived from these supposed nutritive particles. Thus when the nucleoli first appear they are most frequently in contact with the nuclear membrane; and this shows that they are formed at the periphery of the nucleus, and only later come to occupy a central position within it. And since the nutritive particles are usually very numerous in the immediate vicinity of the nucleus, we may conclude that the nucleoli are formed from substance of these nutritive particles, which has been taken up by the nucleus. In the smallest nuclei alone do more than one nucleolus appear, so that the nutritive substance would seem to be taken into the nucleus from several points on its periphery, and then subsequently these several assimilated portions of nutritive substance may fuse together and so produce a single large nucleolus. Accordingly, the substance of the nucleolus would in this way appear to have an extranuclear origin. That these nutritive particles are being successively absorbed by the nucleus is shown by the fact that the increase in the size of the nucleus and of the nucleolus go hand in hand. On the other side, these nutritive bodies in the cytoplasm cannot be considered to be of nucleolar origin, since they usually make their first appearance in the cell body before a nucleolus arises in the nucleus; and if they did have a nucleolar origin, *i.e.*, if they were excreted portions of the nucleolus,

we should expect to find the largest nucleoli in the smallest cells and the smallest ones in the largest cells. Further, the nucleolar substance cannot be regarded as a secretion of the nucleus itself, since this would leave unexplained the peripheral position which it at first occupies in the nucleus. Thus the mode of origin of the nucleolus in these cells would seem to be similar to that of the nucleoli in the ova of the nemerteans. A final point may be noted: the nucleolus accepts the same stains, though more intensely, than do the nutritive particles in the cytoplasm; accordingly, the substance of those bodies which have been absorbed by the nucleus, and then by their fusion in the nucleus produce the nucleolus, must have undergone either a slight chemical or physical change within the nucleus.

The largest mesenchym cells of the pseudoepithelia probably represent the youngest stages of the ova, though in the single individual of this species at my disposal no gonads were present, so that I can bring no proof positive that this is the mode of origin of the egg cells. In *Carinella* it is from similar cells that the genital products are derived, as I have previously shown ('96). Coe ('95) described certain of the more mature egg stages.

In my earlier paper on these cells (*l.c.*) I termed all the nuclear divisions of these cells "amitotic." But renewed study of these elements shows that only the divisions of those cells are amitotic (Figs. 316a and 317), from which the free mesenchym cells are produced. Whereas, in the nuclear divisions of the cells of the pseudoepithelia from which the masses of larger cells are derived I now find evidences of regularity in the distribution of the chromatin, so that probably these divisions are mitotic. However, in these small nuclear divisions it is almost impossible to decide whether we have to do with mitoses or with amitoses without the use of better lenses than those which were at my disposal.

### 21. *Ganglion Cells of Nemerteans.*

I may here briefly mention the relations of the nucleoli in these cells, and for other details refer to a previous contribution of mine ('97).

*Lineus gesserensis.*—Cells of the first type: one or two small nucleoli. Cells of the second type: one nucleolus. Cells of the third type: a single nucleolus, or two of unequal size.

*Cerebratulus lacteus.*—Cells of the first type: as in the preceding species. Cells of the second type: one or two nucleoli. Cells of the third type: one or two nucleoli, which in one case stained differently. Cells of the fourth type: usually one peripheral nucleolus; rarely are two present, and then they are unequal in dimensions.

In all these cells the nucleolus is comparatively small, homogeneous, and no evidences of nucleolar division were seen.

## IV. GENERAL COMPARISONS AND CONCLUSIONS.

Here I shall summarize merely the results of my observations on the nucleolus, and compare them with the conclusions of other investigators. Numerous other morphological points have been brought up, however, in the preceding pages, such as yolk development, differentiation of ova, nuclear divisions, distribution of the chromatin elements in the germinal vesicle at different stages in the growth period, changes in the structure of cytoplasm, etc.

### 1. *Chemistry of the Nucleolus.*

I have made no special chemical study of these structures, except what may be learned from their reactions to stains. In the gregarines no substance could be demonstrated which chemically corresponds to the chromatin of the metazoan cell;[1] but the following table represents the mode of staining of true nucleoli in the somatic and germ cells of the *Metazoa:*

| Stain. | Nucleolus. | Chromatin. |
|---|---|---|
| Del. or Ehrl. haematoxylin, eosin | red | blue. |
| Ehrlich-Biondi stain | maroon or red | green. |
| Acid carmine, nigrosine | blue or greenish | red. |
| Del. haematoxylin, cochineal | pink or red | blue. |
| Safranin, gentian violet, orange | yellow | blue. |

Schwarz ('87) distinguishes in plant cells pyrenin, the substance of the true nucleoli, from the other nuclear substances

[1] That is, not to chromatin in the form of pure nucleic acid.

and finds that it has a closer chemical affinity to the substance of the nuclear membrane (amphipyrenin) than to any other substance. Judging merely from the reactions of these two substances to stains I would agree in this point with Schwarz. Zacharias ('82) shows also for plant cells that the nucleolar substance is *sui generis* and is allied to plastin. O. Hertwig ('92) terms the nucleolar substance "Paranuclein" and observes: "Nuclein und Paranuclein betrachte ich als die wesentlichen Substanzen des Kerns. . . . Beide scheinen mir in irgend welchen Beziehungen zu einander zu stehen." But it is important to note that the true nucleolar substance probably has no chemical relation to the true chromatin (nuclein). Thus karyosomes should not be considered as a particular group of nucleoli, since they are not nucleoli at all, but nodal points of the chromatin reticulum. The substance of every true metazoan nucleolus apparently differs chemically from the chromatin, linin, paralinin, and oedematin (lanthanin); and accordingly "pyrenin" is a term preferable to "paranuclein," though "pyrenin" may include divers substances.

There are also chemical differences between the nucleoli proper ("Hauptnucleoli") and the paranucleoli ("Nebennucleoli"), which occur together in many ova and in a few somatic cells; the substance of the paranucleoli stains more lightly than that of the nucleoli proper. List ('96) distinguishes three kinds of true nucleoli, from a chemical standpoint: (1) the nucleolus of somatic cells; (2) the nucleolus proper of germinal vesicles; and (3) the paranucleolus of germinal vesicles; and he considers the substance of the paranucleus of the germ cell to be closer related chemically to the nucleolus of somatic cells than either of them is to the nucleolus proper of ova. List promises a more complete paper on this subject. The so-called "nucleoli," which react like chromatin, are of course not true nucleoli, but either *karyosomes* (thickened nodal points of the chromatin reticulum) or *chromatin nucleoli* (independent lumps or spheres of chromatin). It is my intention to devote a special paper to the consideration of the latter structures. Other papers on the chemistry: Macallum ('95), Michel ('96), Carnoy and Lebrun ('97).

### 2. *Number of Nucleoli.*

As Flemming ('82) has stated, the number of nucleoli is small in most cells, not more than from one to five. But in certain stages of some cells there may be several hundred (ova of *Reptilia*, *Amphibia*, *Selachii*, nemerteans, subcuticular gland cells of *Piscicola*). Even in those cases just mentioned, where the number of the nucleoli is very large, the immature cell contains only one or a few nucleoli, so that the large number is attained only when the nucleus has increased in size, *cf.* the observations of Auerbach ('74a). Among somatic cells a large number of nucleoli is much more infrequent than among egg cells. At a given stage of a given cell of any one species of metazoan the number of nucleoli is pretty constant, and there is less variability in the number among those cells where the typical number of nucleoli is a small one than in those where a large number is present. In cells where the usual number of nucleoli is one or two, as in those of the nidamental gland of *Montagua*, three may quite frequently be found, but no cells are found in which not a single nucleolus occurs ; in other words, there is in most cases some degree of variability in the number of the nucleoli, and the amount of this variability stands in a more or less direct ratio to the number of the nucleoli, but it is numerically progressive as a rule, tending to produce more than the normal number, and in no cases where cells normally contain nucleoli do we find a regressive numerical variation leading to the total disappearance of nucleoli. In certain few cells no nucleoli are present, and this is the case in more cells than Flemming ('82) was disposed to admit, since not only are specialized cells like mammalian blood corpuscles without them, but they are also absent in certain connective-tissue elements of nemerteans, and in certain other cells of a low degree of vitality.

Auerbach ('90) formulated the law that the number of nucleoli is more or less constant for all the cells of a given species. But this conclusion is certainly erroneous, since in *Doto* there is one nucleolus found in the blood corpuscles and in the ovum, from one to five in the ganglion cells, from one to three in the cells of the nidamental gland, and in the giant cells as many as

forty; and in *Piscicola*, usually one in the ovum and the ganglion cells, about twelve in the mature muscle cells, and three hundred or four hundred in the subcuticular gland cells. From the data at hand we accordingly conclude that the number of nucleoli is not constant for the species. (On the number of nucleoli at different stages in amphibian ova, *cf.* Carnoy and Lebrun, '97a).

In order to determine whether the number of nucleoli in egg cells were fixed for, or in any way determined by, the particular groups of *Metazoa*, I have compiled the following tables (pp. 501–505) for the larger groups, these tables representing the data of previous investigators and of my own observations. In them four classes of germinal vesicles are distinguished according to differences in the number and kind of the nucleoli; this classification is only for convenience' sake, only arbitrarily chosen, and is probably not a natural one. On the left hand is given the name of the genus or group; the asterisk corresponding to each form indicates by its position in a particular vertical column the nucleolar relations of the ovum of the form specified; and next to the asterisk is placed the name of the authority. In some cases two investigators may have reached different conclusions in regard to the nucleolar relations, so that for these cases two asterisks were employed.

One must be extremely cautious in any attempt to draw conclusions from these data, not only because the data are so meager, but also because where data have been culled from so many different observers some of the facts may ultimately prove to have been erroneous. Thus many of these ova may have been examined at only one point in their development, and in others paranucleoli may have been entirely overlooked, or may have been confused with true nucleoli. But taking this mass of observations as it stands, the following general conclusions may be drawn: we find that a large number of nucleoli is not always characteristic of ova with a considerable amount of deutoplasmic substances, for a single nucleolus is typical for the birds and for many of the *Arthropoda*. Further, the number of the nucleoli does not seem to be dependent upon the amount of yolk, nor upon the mode of cleavage,

| Form. | A Single Nucleolus. | More than One Nucleolus, All Alike. | Nucleolus and Paranucleolus. |
|---|---|---|---|
| | | *Coelenterata.* | |
| Esperella | * (H. V. Wilson) | | |
| Spongilla | * (Fiedler) | | |
| Tedamione | | * (H. V. Wilson) | |
| Hircinia | | * (H. V. Wilson) | |
| *Hydrozoa* | * (Weismann) | | |
| Aequorea | * (Häcker) | | |
| Hydractinia | * (Bunting) | | |
| Podocoryne | * (Bunting) | | |
| Geryonia | * (Fol) | | |
| Tubularia | * (Doflein) | | |
| Eucope | | * (O. Hertwig) | |
| Aeginopsis | | | * (O. Hertwig) |
| Hydra | | | * (Brauer) |
| Nausithoë | | | * (O. Hertwig) |
| Pelagia | | | * (O. Hertwig) |
| Physophora | | | * (O. Hertwig) |
| Rodalia | | | * (O. Hertwig) |
| *Ctenophora* | * (Chun) | | * (mihi) |
| | | *Plathelminthes.* | |
| Bothriocephalus | | * (Schauinsland) | |
| Distomum | * (Schauinsland) | | |
| *Polycladidea* | * (Lang) | | |
| *Tricladidea* | | * (Jijima) | |
| Rhabdocoele | * (Repiachoff) | | |
| Prorhynchus | * (Vejdovský) | | |
| Bothrioplana | * (Vejdovský) | | |
| Haplodiscus | * (Böhmig) | | |
| | | *Nemertini.* | |
| Carinella | * (Bürger) | | |
| Cerebratulus | * (Hubrecht) | | * (Bürger, Coe) |
| Lineus | * (mihi) | | |
| Malacobdella | | * (v. Kennel) | |
| Drepanophorus | * (Bürger) | | |
| Tetrastemma | | * (mihi) | |
| Amphiporus | | * (mihi) | |
| Stichostemma | | * (mihi) | |
| Zygonemertes | | * (mihi) | |
| Proneurotes | | * (mihi) | |
| Prosadenoporus | | * (Bürger) | |
| Pelagonemertes | | | * (Hubrecht) |

| FORM. | A SINGLE NUCLEOLUS. | MORE THAN ONE NUCLEOLUS, ALL ALIKE. | NUCLEOLUS AND PARANUCLEOLUS. |
|---|---|---|---|
| | | *Annelida.* | |
| Nereis | | | * (E. B. Wilson) |
| Spinther | * (Korschelt) | | |
| Ophryotrocha | * (Korschelt) | | |
| Sternaspis | * (Vejdovský) | | |
| Polydora | | | * (mihi) |
| Spio | | | * (Giard) |
| Capitellids | | | * (Eisig) |
| Polygordius | | | * (Fraipont) |
| *Oligochaeta* | * (Vejdovský) | | |
| Rhynchelmis | * (Vejdovský) | | |
| Lumbricus | | | * (Claparède) |
| Nephelis | * (Leydig) | | |
| Branchiobdella | * (Ludwig) | | |
| Haemopis | * (Ludwig) | | * (O. Hertwig) |
| Piscicola | * (mihi) | | |
| Clepsine | | * (Whitman) | |
| Piscicola | | * (Leydig) | |
| | | *Arthropoda.* | |
| Homarus | * (Herrick) | | |
| Porcellio | * (St. George) | | |
| Oniscus | * (Wielowiejski) | | |
| Heterocope | * (Rückert) | | |
| Diaptomus | * (Rückert) | | |
| Argulus | | * (Leydig) | |
| Astacus | | * (Wielowiejski) | |
| Cyclops | | | * (Häcker) |
| Sida | | | * (Häcker) |
| Canthocamptus | | | * (Häcker) |
| Moina | | | * (Häcker) |
| Euchaeta | | | * (vom Rath) |
| Epeira | * (Korschelt) | | |
| Dolomedes | * (Korschelt) | | |
| Phalangium | * (Korschelt, Leydig) | | * (Henking) |
| Lycosa | * (Leydig) | | |
| Theridium | * (v. Wittich) | | * (Leydig) |
| Tetragnatha | | | * (Leydig) |
| *Araneina* | | | * (Wielowiejski) |
| *Acarina* | | | * (Wielowiejski) |
| Zilla | | | * (Van Bambeke) |

| Form. | A Single Nucleolus. | More than One Nucleolus, All Alike. | Nucleolus and Paranucleolus. |
|---|---|---|---|
| Julus | * (Stuhlmann) | | |
| Geophilus | | * (Balbiani) | * (Leydig) |
| Glomeris | | | * (Stuhlmann) |
| Lithobius | | | * (Leydig) |
| Peripatus | * (Stuhlmann) | | |
| Blatta | * (Brandt) | | |
| Nepa | * (Will) | | |
| Notonecta | * (Will) | | |
| Carabus nemoralis | * (Stuhlmann) | | |
| Gryllotalpa | * (Stuhlmann) | | |
| Pieris | * (Stuhlmann) | | |
| Anabolia | * (Stuhlmann) | | |
| Bombus | * (Stuhlmann) | | |
| Anomalia | * (Stuhlmann) | | |
| Ophion | * (Stuhlmann) | | |
| Ephialtes | * (Stuhlmann) | | |
| Pemphigus | * (Leydig) | | |
| Musca | * (Wielowiejski) | | * (Stuhlmann) |
| Necrophorus | | * (Stuhlmann) | |
| Geotrupes | | * (Stuhlmann) | |
| Banchus | | * (Stuhlmann) | |
| Pimpla | | * (Stuhlmann) | |
| Stenobothrus | | * (Leydig) | |
| Meloe | | * (Leydig) | |
| Libella | | | * (St. George) |
| Melolontha | | | * (Wagner) |
| Lina | | | * (Stuhlmann) |
| Lycus | | | * (Stuhlmann) |
| Sphinx | | | * (Stuhlmann) |
| Ambyteles | | | * (Stuhlmann) |
| *Echinodermata.* | | | |
| Psammechinus | * (Bergh) | | |
| Echinocardium | * (Bergh) | | |
| Echinus | | | * (Häcker) |
| Toxopneustes | | | * (Häcker) |
| Asteracanthion | | | * (O. Hertwig) |
| Sphaerechinus | | | * (O. Hertwig) |
| Amphidetus | * (Ludwig) | | |
| Solaster | * (Ludwig) | | |
| *Mollusca.* | | | |
| Chaetoderma | * (Wirén) | | |
| Proneomenia | | | * (Hubrecht) |

| Form. | A Single Nucleolus. | More than One Nucleolus, All Alike. | Nucleolus and Paranucleolus. |
|---|---|---|---|
| Neritina | * (Blochmann) | | |
| Doto | * (mihi) | | |
| Montagua | | | * (mihi) |
| Tellina | | | * } (O. Hertwig) |
| Helix | * (Platner) | | * } |
| Limax | | | * (Mark) |
| Arion | | | * (Platner) |
| Doris | | | * } (Lönnberg) |
| Aeolidia | | | * } |
| Amphorina | | | * (Trinchese) |
| Paludina | | | (Leydig) |
| Anodonta | | | * (Flemming) |
| Unio | | | * (Hessling) |
| Mytilus | | | * (Lönnberg) |
| Pholas | | | * (List) |
| Cyclas | | | * (Stepanoff) |
| | *Tunicata.* | | |
| Distaplia | * (Davidoff) | | |
| Phallusia | * (Bergh) | | |
| Botryllus | * (Pizon) | | |
| Clavelina | * (Seeliger) | | |
| Ciona | | | * } (Floderus) |
| Styelopsis | | | * } |
| Ascidia | | | * (O. Hertwig) |
| | *Vertebrata.* | | |
| Amphioxus | * (Van d. Stricht) | | |
| Petromyzon | * (Böhm) | | |
| Scyllium | | * } (Rückert) | |
| Torpedo | | * } | |
| Pristiurus | | * } | |
| Scorpaena | | * (Van Bambeke) | |
| Conger | | * } (Scharff) | |
| Gadus | | * } | |
| Trigla | | * } | |
| Gasterosteus | | * (Ransom) | |
| Anguilla | | * (Brock) | |
| Cyprinus | | * (Eimer) | |
| Rana | | * (Born) | |
| Amblystoma | | * (Fick) | |
| Triton | | * (Leydig) | |
| Emys | | * (mihi) | |
| Lacerta | | * (Eimer) | |
| "Turtle" | | * (Agassiz) | |

| Form. | A Single Nucleolus. | More than One Nucleolus, All Alike. | Nucleolus and Paranucleolus. |
|---|---|---|---|
| Gallus | * (Holl) | | |
| Fringilla | * (v. Wittich) | | |
| Columba | * (mihi) | | |
| Felis | * (St. George) | | |
| Cavia | * (Rein) | | |
| Mus | * (Holl) | | |
| Vespertilio | * (v. Beneden) | | |
| Sus | * | | |
| Myoxus | * (Leydig) | | |
| Talpa | * | | |
| Ovis | | | |
| Lepus | | | * (St. George) |
| Homo | * (Nagel) | | * (Flemming) |

nor yet upon the mode of deposition of the egg (*i.e.*, whether it is pelagic, hatched in a cocoon, or nourished in an uterus). These facts hardly warrant an attempt to explain the factors limiting the number of nucleoli, and perhaps such explanations should rather be expected from experimental workers than from purely structural observers. On examining the metazoan groups in detail we find in certain of them a degree of uniformity in regard to the number of nucleoli. Thus the only vertebrate ova with two kinds of nucleoli are those of *Lepus* and *Ovis*. A single nucleolus is the rule for *Amphioxus*, *Petromyzon*, the birds, and most of the mammals; the *Reptilia*, *Amphibia*, *Teleostii*, and the *Selachii* have numerous nucleoli. In the *Tunicata* there is either a single nucleolus or a nucleolus and paranucleoli; this is also the rule for the *Echinodermata*, *Mollusca*, and *Annelida*. In the *Arthropoda* there is considerable diversity in regard to the number and differentiation of the nucleoli. In the nemerteans we find most usually either a single nucleolus or a large number of small ones. In the *Plathelminthes* one or two is the rule; this is also most frequently the case for the coelenterates, but in some of the latter paranucleoli have been described.

### 3. *Position of the Nucleolus in the Nucleus.*

Where a single nucleolus is present it almost always lies excentrically, though not against the nuclear membrane. Those cases where it regularly occupies the center of the nucleus must be regarded as exceptional ; thus I am unable to agree with Macfarlane that the nucleolus is either the morphological or the tropic center of the cell. At the time of its origin, and often at the time of mitosis, the nucleolus may be in contact with the nuclear membrane. Where a number of nucleoli are present they may be scattered irregularly through the nucleus, or grouped at one point in it, or be concentrically arranged ; their position is often dependent upon the stage of the development of the nucleus. Thus in the metanemerteans examined by me they lie at the periphery in the smallest germinal vesicles, then wander towards its center, and finally migrate to the periphery again.[1]

The nucleoli lie in the nuclear sap, as a rule not in any close connection with the chromatin reticulum. But in those cases where the nucleolus may be unusually large it appears to be suspended by the fibers of this reticulum, but not in such a way that the fibers penetrate into its substance, but become simply wound around its surface ; thus it appears that when the nucleolus increases in size it forces apart the fibers of the nuclear network in such a way that the latter gradually produce a latticework on its surface. In this way the nucleoli may be more or less held in position in the nucleus, but Herrick's observations on the gravitation of the nucleolus show that it is not firmly held by the chromatin fibers. The nucleolus is, as it were, a ball lodged in the branches of a tree, its movements hindered by the intervening branches, but nevertheless not immovable. Various views on the mode of suspension of the nucleolus : Pflücke ('95), Heidenhain ('92), Rosen ('95), Jensen ('83), Zimmermann ('96). Note also its peculiar position in *Synapta* (Leydig, '52).

[1] For the opinions of other authors, *cf.* the reviews of the papers of Pflücke ('95), Heidenhain ('92), Rosen ('95), Jensen ('83), Leydig ('52), Zimmermann ('96), Schneider ('91).

### 4. *General Morphological Structure of the Nucleolus.*

The ground substance of the nucleolus is more or less dense, but not brittle, and either homogeneous or finely granular, rarely coarsely granular. It may be either fluid or viscid in consistency.

In the greater number of cases it has no limiting membrane. Such a membrane was found by me only in the germinal spot of *Polydora*, and here it appeared to be merely a denser portion of the ground substance. When any small nucleolus is viewed in its totality a membrane appears to surround it, but this phenomenon is due to the refraction of light from its convex surface, and many observers have been misled by this appearance into supposing that a membrane is present. Others in describing those states of nucleoli in which a large vacuole is present have erroneously described the peripheral layer of true ground substance as a nucleolar membrane ; it is necessary to distinguish between such a peripheral layer, which consists of true ground substance, and a nucleolar membrane proper, which is a differentiation of the ground substance. Some authors, *e.g.*, Lavdowsky ('94), have described a membrane of chromatin enveloping the nucleolus, and I have found that those of the giant cells of *Doto* may sometimes be surrounded by a mass of chromatin. But this apposition of a mass of chromatin in *Doto* is certainly an artefact, though it would seem probable that the nucleolus in some cases has an envelope of chromatin forming a distinct capsule separated from the chromatin network of the nucleus. I am able, however, to corroborate the observations of Macfarlane ('81) and Pennington ('97), that the nucleolus in *Spirogyra* has a true membrane.[1]

A very unusual structure of the nucleolus is that afforded by the salivary gland cells of *Chironomus* as described by Balbiani ('81), Leydig ('83), Korschelt ('84), and Macallum ('95). C. Schneider ('91) supposes the nucleoli, as well as the rest of the nuclear substance, to consist of "Gerüst" (linin?) and chro-

[1] The following writers have described nucleolar membranes: Macallum ('95), Carnoy and Lebrun ('97a), Will ('85), Holl ('93), Roule ('83), Bürger ('90), Ogata ('83), Vejdovský ('82), Meunier ('86), Carnoy ('86), Mann ('92).

matin, and considers the nucleoli to be only isolated masses of chromatin surrounded by linin sheaths ; these observations have not been corroborated by any other writers and would seem to be due to faulty methods of fixation.

In opposition to Meunier ('86), and in agreement with most investigators, I must conclude that vacuoles are normal structures in nucleoli, since they may be seen after the most diverse methods of fixation, and their size and number are not only to some extent limited for the particular cell, but are also different at different periods in the metamorphoses of the nucleus. It is the rule that the youngest nucleoli are homogeneous, and that vacuoles first arise when they have increased in size. Their size and number vary at different phases in the development of the nucleolus. Very frequently a number of smaller ones appear, and then these subsequently fuse together and produce a larger one. The nucleoli of egg cells are characterized as a rule by more numerous or larger vacuoles than those of somatic cells, and in many somatic cells these vacuoles appear to be wholly absent. The vacuolar substance appears in some cases not to be a derivative of the ground substance of the nucleolus, but to be derived from without the nucleolus (ova of *Doto* and *Montagua*). Perhaps this vacuolar substance always has an extranuclear origin, since in many cases a germinal spot grows larger merely by an increase in its volume, while the ground substance seems neither to increase nor diminish.

The alveolar structure of nuclei as described by Purcell ('94), Schaudinn ('94), Korschelt ('95), and Lauterborn ('95b) is probably referable to the regular distribution of equal-sized vacuoles in the nucleolus.

A "Kernkörperchenkreis," a shell of minute granules arranged concentrically around the nucleolus, has been described by Eimer ('71, '72), Auerbach ('74a, who considered it to be the result of opposing repulsive forces of the nucleolus and nuclear membrane), Brass ('89), Pflücke ('95), Platner ('89a), Smirnow ('90), Engelmann ('80), Carnoy and Lebrun ('97a). A more or less similar phenomenon has been described by me for ganglion cells of *Doto*. Such a nucleolar circlet must be considered, in most cases at least, an artefact. But in this cate-

gory should not be classed small masses of nucleolar substance grouped around a larger one, these being normal phenomena during the growth period of nucleoli.

Reticulations within the nucleolar substance have been described by some few authors. Thus Carnoy ('85, '97a), Meunier ('86), and Moll ('93) described nucleoli containing a skein of chromatin, but Zacharias and Strasburger ('88) did not find anything resembling these supposed skeins described for *Spirogyra*. Leydig ('88) states that the germinal spot of *Lycosa* "bietet das Bild eines Knäuels dar." Fromann ('84) described the nucleolar substance as consisting of granules connected by fibers, Bütschli ('80) found the nucleoli of *Dinoflagellata* to contain a fine reticulum, and Davidoff ('89) states that the germinal spot of *Distaplia* takes up portions of the nuclear reticulum into itself (but *cf.* Bancroft, '98, and Shäfer, '80). The only structure which was found by me to resemble a skein was present in the later stages of the germinal spot of *Polydora;* but in this object, owing to the gradual confluence of the vacuoles, which thus produce anastomosing channels of vacuolar substance in the ground substance of the nucleolus, it is the true ground substance which represents a skein-like appearance. It is very probable that Carnoy and his followers have mistaken the vacuolar substance for the ground substance, and have considered the true ground substance to be chromatin; I am forced to conclude that in all probability there are no skeins of chromatin lying in any metazoan nucleolus, since I have never found any evidence of chromatin in it in any metazoan cell. But it is not improbable that in the nuclei of gregarines chromatin may be massed in some or all of the nucleoli.

Nucleolini, granules within the nucleolus, have frequently been observed. A single nucleolinus to a nucleolus has been described by Vejdovský ('95b), Morgan ('96), Agassiz ('57), Kleinenberg ('72), Leydig ('88), Macfarlane ('85), Lavdowsky ('98), A. Brandt ('78), Van Bambeke ('86), Kosinski ('87, '93); several nucleolini to a nucleolus, by Bürger ('90), Rhumbler ('93), Holl ('93), Wolters ('91), Schrön ('65), Scharff ('88), Seeliger ('82), Gjurasin ('93), Haeckel ('74), Mann ('92), Van Bam-

beke ('97b), Mark ('77), Bancroft ('98). Compare also the following: Huie ('97), Van Bambeke ('97), Kosinski ('87, '93), Mark ('77), Zimmermann ('96), Hodge ('94). I have found these bodies occurring in varying number, though most frequently absent, in the nucleoli of various cells, and they appeared to be merely loosened portions of the ground substance which had come to lie within a vacuole. Macfarlane and his pupil Mann have described nucleolini under the names "endonucleolus" and "nucleolo-nucleus" as occurring singly and with great constancy in certain plant cells, though Zacharias ('85) studied Macfarlane's object (*Chara*) and makes no mention of any of these structures. Macfarlane ascribes the utmost importance to his "endonucleolus," regarding it as the tropic center of the cell and as an important mechanical agent during nuclear division. Mann has not only described a most complex structure of the nucleolus, such as no other observer has yet seen, but also has found fine fibrils radiating out from it, which he supposes to penetrate through the nuclear cavity. From my own observations, and in agreement with the majority of observers, I can attach no particular morphological significance to the nucleolinus; it appears to be only a detached portion of the nucleolar ground substance, to be in most cases absent, and when present to vary greatly in regard to size, position, and number. It is undoubtedly the case that many structures which have been described as nucleolini are in reality minute vacuoles, which from their refrangibility appear to be granules; such is the case with the minute vacuoles of *Polydora* and *Montagua* when studied after the action of certain stains, and has been shown for other objects by Zimmermann and Huie, Lavdowsky found in the nucleolus a central vacuole, and in the latter a small granule, which he supposed to be "das noch in Entwicklung begriffene Centrosoma," destined to finally pass out of the nucleolus; he was unable to determine how it does wander out of the nucleolus and become the centrosome, so that his suggestion has merely the value of a hypothesis. Van Bambeke describes the nucleolinus of the germinal spot of *Amaurobius* as "doué d'un mouvement très vif"; this interesting phenomenon certainly deserves investigation, though

it is not impossible that the supposed nucleolinus was in reality a microörganism inclosed in the vacuole of the nucleolus. (*Cf.* also Flemming's observation on the egg of *Ascidia*, '97.) Supposed nerve fibrils in the nucleolus have been described by Eimer ('73, '90).

### 5. *Polarity of the Nucleolus.*

In the gregarine (*Gonospora?*) from the intestine of *Lineus gesserensis* it is the rule that the vacuoles make their first appearance at that pole of the nucleolus which is nearest to the nuclear membrane. In the germinal spot of *Montagua* the opposite position of the large excentric vacuole is the rule, though the percentage of cases in which the vacuole has a particular position with regard to the nuclear membrane is less than in the gregarine. On the contrary, in the germinal spots of *Piscicola* and *Rodalia* there is no regularity in regard to the position of the vacuoles, and in that of *Polydora* the vacuoles are, at the time of their first appearance, usually central in position. In the germinal spots of many other *Metazoa*, where a single large vacuole is present it more usually lies excentrically than centrally, though its position appears to be independent of the proximity of the nuclear membrane ; so that in these cases we can speak of a certain polarity in regard to the position of the vacuole within the nucleolus, and not of a polarity of the axis of the nucleolus in regard to the position of the nuclear membrane. But in the two gregarines examined by me the substance of the nucleolus, or of some of the nucleoli, is differentiated at two poles of the nucleolus, so that the portion of the ground substance at one end stains differently from that of the other end of the nucleolus ; this state apparently does not occur in the nucleoli of metazoan cells. It remains to be solved whether in the gregarines the chromatin or its physiological equivalent is localized at some particular point or pole of the nucleolus, *i.e.*, whether or not such nucleoli should be compared to the nucleoli of the *Metazoa*.

6. *Amoeboid Movements, Divisions, and Fusions of Nucleoli.*

Amoeboid movements have been seen in life in metazoan cells by the following observers (germinal vesicles): A. Brandt ('74, *Blatta*), Eimer ('75, *Silurus*), O. Hertwig ('76, *Rana*, *Pterotrachea*), La Valette St. George ('66, *Libella;* '83, *Isopoda*), Bergh ('79, *Gonothryaca*), Van Beneden ('69, '76, *Polystomum*, *Rana*), Balbiani ('64, several genera of spiders), Leydig ('83, *Libella*), A. Brandt ('78, numerous *Insecta*, *Distomum*), Van Bambeke ('86, *Blatta*), Knappe ('86, *Bufo*), Auerbach ('74a, *Teleostii*). In somatic cells: Schwalbe ('76, sympathetic ganglion cells of *Rana*), Kidd ('75, epithelial cells from the mouth of *Rana*), Hodge ('94, nerve cells of *Rana*), Auerbach ('74b, salivary gland cells of *Musca*). In *Protozoa:* Van Beneden ('69, '76, *Gregarina*, *Monocystis*). In plants, Zacharias ('85) has observed amoeboid movements in the nucleoli of *Chara* (an observation overlooked by Zimmermann, who states that such movements have not been seen in plants).

These observations would show that amoeboid movements are probably natural phenomena of certain nucleoli, but one should not be too positive of the naturalness of these phenomena, since some of the observations were made upon the heated stage, and in all of them the object was probably more or less compressed and placed in artificial conditions. But they are in all probability frequently normal phenomena, since, as we shall see, divisions and fusions of nucleoli are certainly normal and of wide occurrence, and the latter can only be classed as forms of amoeboid motion. The question arises, Are these movements wholly passive, caused by movements in the other parts of the nucleus, or should they be considered an inherent function of the nucleolus? The latter alternative would seem the more probable, since no movements of the other nuclear elements are known in the resting cell. Van Beneden ('69) has described rhythmic expansion and contraction of the volume of nucleoli in gregarines. But all these movements of nucleoli should not be regarded as automatic motions of the nucleolus in the sense that an *Amoeba* forms and retracts processes; but rather with Rhumbler ('93) they should be regarded as "Auf-

lösungsvorgänge," due to chemical changes in its substance. *Cf.* the movements described by Flemming ('97) for the ovum of *Ascidia.*

The nucleolus has in some cases a viscid consistency (as described by me for *Stichostemma*) and then may be irregular in form ; in other cases it is more fluid, and this is probably the case when it has regularly a spherical shape, *i.e.*, the globular form characteristic of drops of a thin liquid. Its more or less fluid consistency allows changes of form, division into particles, and fusions of neighboring nucleoli.

The division of a nucleolus into two or more parts is a normal and regular phenomenon in many cells, though all nucleoli do not show this property. Two kinds of nucleolar division may be distinguished : (1) that mode by which the nucleolus becomes elongated and then breaks into two or more parts, whereby the daughter-nucleoli are usually capable of further division ; and (2) that mode by which the nucleolus fragments nearly simultaneously into a number of small granules. From my own observations the former mode is evinced by the nucleoli of the muscle and giant gland cells of *Piscicola*, the giant cells of *Doto*, and the germinal spots at certain stages in the ovogenesis of the metanemerteans. This mode of division cannot be regarded as a phenomenon of nucleolar degeneration, since the nucleolus and its products may often continue to increase in size during the process of division. But the second mode, that by which the nucleolus breaks into a large number of granules, since it is particularly characteristic of the nucleolus in nuclear division, may be regarded as a process of degeneration ; the case of divisions during nuclear division shall be considered later. A strange mode of nucleolar division has been described by A. Schneider ('83). According to his observations on *Klossia*, the smaller nucleoli are portions of the inner substance of the larger nucleoli and wander out of each larger one by passing through the pore ("canal micropylaire ") of the cortical substance of the latter; this intranucleolar origin of the smaller nucleoli is still open to question, since it was not observed in life, and since the canal micropylaire was observed in only one nucleolus. Marshall ('92) has described

a somewhat similar method of formation of the smaller nucleoli of *Gregarina blattarum*. Now I found in the nucleus of the gregarine from *Lineus* numerous nucleoli of different dimensions, and often irregular in their outlines ; and this irregularity in form would point not only to amoeboid movements of the nucleoli, but also to nucleolar divisions, since in the largest nuclei we find a large number of small nucleoli. All appearances showed that these smaller nucleoli are division products of the larger ones ; but it seems that they simply bud off from the surface of the latter, and are not preformed in their interior. In other words, Schneider and Marshall are probably correct in concluding that the smaller nucleoli are disassociated portions of the larger ones ; but they may perhaps be mistaken in assuming that they are preformed in the interior of the latter, since these investigators may have mistaken vacuoles for intranucleolar nucleoli. (Other observations on nucleolar divisions in resting cells : Hermann, '89 ; Vejdovský, '95a ; Bütschli, '80 ; R. Hertwig, '76 ; Kultschitzky, '88 ; Bergh, '79 ; Bannwarth, '92 ; Stuhlmann, '86 ; A. Brandt, '78 ; Scharff, '88 ; Eisig, '87 ; Cunningham, '95 ; Kosinski, '87, '93 ; Carnoy and Lebrun, '97a ; Steinhaus, '88 ; Cuénot, '91 ; Metzner, '94.)

Fusions of nucleoli are not as widely known as divisions, but there are some facts which would show that the former processes are by no means unusual in their occurrence. Such fusions have been described for cells of plants by Zacharias ('85), Mann ('92), and Wager ('93) ; for animal cells by Rhumbler ('93, '95), Brauer ('91), Leydig ('50), Pfitzner ('83), and Rückert ('92). I have found fusions of the nucleoli to be characteristic phenomena of certain stages in the maturation of the germinal vesicles of nemerteans, an extreme case being furnished by *Stichostemma*, where sometimes all the nucleoli may fuse together at the center of the nucleus, and so produce a single large one. The nucleolus at the time of its origin may be said to be undergoing a process of fusion, since it is produced by the coalescence of numerous smaller portions of nucleolar substance. There is nothing problematical in regard to the fusion of nucleoli, since it is a physical property for bodies of like nature (when fluid) to fuse together when they come into contact, though this

process is to some extent dependent upon the nature of the medium in which they are suspended (*cf.* Rhumbler, '93). (*Cf.* also Hermann, '89b ; Bouin, '97 ; Mertens, '93 ; Debski, '97 ; Carnoy and Lebrun '97a ; Koernicke, '96.)

### 7. *Paranucleoli and Pseudonucleoli, Double Nucleoli, etc.*

The term paranucleolus is here adopted as equivalent to Flemming's "Nebennucleolus," and I shall use simply the name "nucleolus," or "nucleolus proper," instead of "Hauptnucleolus." E. B. Wilson's terms, "principal nucleolus" and "accessory nucleolus," are somewhat inconvenient on account of their length, and may be misleading, since the "principal nucleolus" is often smaller than the "accessory nucleolus." "Paranucleolus," as used here, is not employed in the same sense as by Stuhlmann ('86), since he expresses by this term portions of the nuclear reticulum ; in my paper the term "nucleolus" has not been used for any part of the chromatin elements of the nucleus.

In many egg cells, especially those of the *Mollusca*, *Annelida*, *Tunicata*, and *Echinodermata*, two kinds of nucleoli occur according to the writers on these objects, which differ from one another chemically and in some cases also structurally ; these are the nucleolus proper and the paranucleolus. Of these it is the nucleolus proper which seems to be morphologically comparable to the nucleoli of somatic cells, however the two may differ chemically. The paranucleolus may be either larger or smaller than the nucleolus, and appears usually to be distinguishable from the latter by staining less deeply with the specific nucleolar stains. In the spermatoblast of the mouse these two kinds of nucleoli have been found by Hermann ('89) ; and in somatic cells by Lönnberg ('92, liver cells of *Doris*, *Polycera*, *Aeolidia*, and *Astacus*) ; perhaps the smaller of the two nucleoli found by me in the blood corpuscles of *Doto* might represent a paranucleolus. In plant cells apparently only one kind of nucleolus is present, this being comparable morphologically to the nucleolus proper of the germ cells and to the nucleoli of the somatic cells of *Metazoa*. Thus paranucleoli are quite

frequent in many egg cells, infrequent in somatic cells of the *Metazoa*, and apparently never present in plant cells. In each such egg cell there may be either one nucleolus proper and from one to several paranucleoli (this being the most usual case), or there may be a single paranucleolus and a few nucleoli proper. In the ova of three forms examined by me there were two kinds of nucleoli present, namely, in *Montagua*, *Polydora*, and *Rodalia*. In my descriptions I have employed the term "pseudonucleolus" for these secondary nucleoli, since in this form they have a different structure from that of the nucleolus proper, but nevertheless stain in the same way, so it is difficult in this case to decide whether they correspond to paranucleoli, and hence I have used the indifferent name "pseudonucleoli" for them. In *Polydora* we found from one to three paranucleoli in the larger germinal vesicles, and these are always apposed to the nucleolus. Then the smaller, deeply staining bodies in the maturer stages of the ovum of *Rodalia* may be comparable to paranucleoli. Whether the remarkable structures of the germinal vesicles of *Tetrastemma catenulatum* are paranucleoli, I am wholly unable to decide. This problem of different types of true nucleoli in the same nucleus is one of the most difficult in the study of nucleolar structures, so that it is necessary to discuss it more in detail.

A. Schneider ('83), Brauer ('91), and Floderus ('96) consider the paranucleoli to be derivatives of the nucleolus proper, more especially to be buds from its surface. Häcker ('93a) considers them to be secretions of the chromatin. Flemming ('82) doubts whether "die Unterscheidung von Haupt- und Nebennucleolen eine durchgehende Geltung beanspruchen kann"; he finds that in *Anodonta* the two are at first in contact, but that later they become separated. Giard ('81) finds in the ovum of a Spionid one nucleolus, and later there appears in the nucleus a much smaller body, which fuses with the former. Lönnberg ('92) thinks that the paranucleoli may serve for the acquisition of nourishment, or may contain reserve nourishment. List ('96) considers that the paranucleoli and the nucleoli of the somatic cells are more closely allied to one another than to the nucleolus proper of the ova, and that the former two "mindestens

verschiedene Modificationsstufen des Paranucleins . . . darstellen." Hessling ('54) found that in the ovum of *Unio* the smaller paranucleolus is divided off from the larger nucleolus proper. Häcker, in his last paper on the subject ('95), considers that the paranucleoli are of later formation than the nucleolus proper.

Now in many of those cases where a paranucleolus and a nucleolus have been described lying in contact with one another it is very probable that the vacuolar portion of the vacuole has been described as a paranucleolus. I have no doubt that many of the earlier observers, who studied the nucleolus mainly in the living egg, have been thus misled, since only sections of nucleoli can show the true nature of the nucleolus. Thus Lönnberg, in speaking of "helle Kugeln" in the germinal spot of *Mytilus*, says: "Es ist schwer zu entscheiden, ob es sich hier nur um Vacuolen handelt"; and any one studying the unsectioned nucleolus of *Montagua* would be misled into supposing that here two nucleoli of different consistency are apposed to one another. Accordingly, we must be very careful in treating as facts some of the observations of the earlier workers, which were made upon unstained and unsectioned material.

But there are undoubtedly many cases in which two kinds of nucleoli do occur,[1] and this is especially so in germinal vesicles. The nucleolus and the paranucleolus may be in contact with one another, may be always separated, may at first be in contact and later become separated, or finally may be at first separated and later come into mutual contact. Are these paranucleoli derived from the nucleolus proper, or have they a distinct origin? In the ovum of *Polydora* the paranucleoli appear towards the close of the maturation period, and then are always in contact with the outer surface of the nucleolus proper.

[1] *Cf.* the reviews of the following papers: Floderus ('96), Hermann ('89a, b, '97), Vejdovský ('95a), Flemming ('74, '82), Häcker ('93a), Kultschitzky ('88), Lukjanow ('87b), Brauer ('91, '92), Nussbaum ('87), Rein ('83), Henking ('87), Van Beneden ('80), Leydig ('55a, '50), Stauffacher ('93), Stepanoff ('65), Giard ('81), Mark ('77, '81), Lönnberg ('92), Stuhlmann ('86), List ('96), Van Bemmelin ('83), Platner ('86), Claparède ('69), Hessling ('54), Rückert ('94), Bouin ('97), Vom Rath ('95b), Moore ('95), Weismann and Ishikawa ('89), Fol ('89), Lacaze-Duthiers ('57), Fauvel ('97), Held ('95), Michel ('96), Steinhaus ('88), Metzner ('94), Braem ('97), Siebold ('39), Reinhard ('82), Kraepelin ('92), Davenport ('91).

In the ova of *Montagua* and *Rodalia* they are never in contact with the nucleolus. In none of these three cases observed by me does there seem to be any genetic connection between the paranucleoli and the nucleoli proper. And in other cases, where the two are separated (this separation is the most usual state), no genetic connection between the two has been described; and even in that smaller number of cases where they are in contact with each other at some period of their development, no positive proof of their genetic relation has been offered. Therefore we might conclude, though with reserve, that in the greater number, if not all, cases the paranucleoli are not derivatives of the nucleolus, but are products *sui generis*. It is the rule that the nucleolus proper appears in the nucleus before the paranucleoli arise, the latter usually arising first towards the close of the growth stages. Accordingly, though I cannot corroborate Häcker's ('95) conclusions as to the origin of the nucleolar substance, I am inclined to agree with him that portions of nucleolar substance are successively deposited in the nucleus, and that those portions which are deposited last, after the nucleus has undergone important physiological and chemical changes, would differ from the portion first produced (that of the nucleolus proper), and so would represent the paranucleolus. And there are certain facts from my own observations which would support this view. In the earlier stages of the maturation of the ovum of *Tetrastemma* and *Zygonemertes* there are a large number of nucleoli produced successively at the periphery of the nucleus; these then wander successively to the center of the nucleus, and then from that point again to the periphery. Now in this last stage, when the nuclear filaments are commencing to arise, we find, usually in contact with the latter, much smaller, more deeply stained nucleoli, and these I have termed "nucleoli of the second generation." We have found, accordingly, that after the nucleus has passed through very marked physiological changes (increase in size, redistribution of chromatin), another kind of nucleoli appears, which may or may not be morphologically compared to the paranucleoli of other ova. These nucleoli of the second generation have neither a genetic nor a physiological

relation to those of the first generation ; and their difference from the latter is probably due to the fact that they have been produced at a time when very different physiological conditions exist in the nucleus.

It is not my intention in this contribution to deal in any detail with those cases where double nucleoli occur in a cell, or those where two chemically and morphologically different kinds of "nucleoli" occur in the same nucleus ; to these cases it is my intention to devote a special study. But preliminarily, from those observations which I have made on this subject, the following conclusions are in order. In a nucleus there sometimes occurs a double nucleolus, the component parts of which may each represent a true nucleolus ; or such a double nucleolus may consist of a true nucleolus apposed to a chromatin-nucleolus (according to my unpublished observations on the spermatocytes of the beetle *Harpalus*). Further, and this is frequently the case in resting spermatocytes of the first order, the nucleus may contain a true nucleolus separated from a chromatin-nucleolus ; and in *Pentatoma*, the account of the spermatogenesis of which will be shortly published by me, the unique process occurs of the chromatin-nucleolus being a metamorphosed chromosome (one of the fourteen chromosomes of the last spermatogonic division becoming the chromatin-nucleolus of the first spermatocyte) ! This peculiar structure of *Pentatoma* divides with the true chromosomes in the first reduction division. In another case where I have been able to follow all the developmental stages of a chromatin-nucleolus, namely, in cells of the hypodermis of the larva of *Carpocapsa*, I found it to originate from one of the granules of the nuclear reticulum, — a particular one of these granules (karyosomes) gradually increasing in size until it attains large dimensions ; during its growth period it is usually attached to one of the true nucleoli of the cell. What is of importance in these two cases (*Pentatoma* and *Carpocapsa*) is the distinction emphasized between the true nucleolus and a karyosome or chromatin-nucleolus : the latter always standing in genetic connection with the true chromatin, while the former, so far as my observations go, is never derived from this substance. These observations are not

wholly out of place in the present paper on the true nucleolus, since they are necessary to prove that the true nucleolus is in all cases never derived from the chromatin ; where " nucleoli " have been described as arising from the chromatin elements of the nucleus, such structures cannot correctly be included under the term " nucleolus," when the latter is used in the proper sense.

### 8. *Relation between Nucleoli and Centrosomes.*

The greater number of cytologists agree that there is no genetic relations between these two structures ; and my observations on the egg of *Piscicola* as well as more recent studies on other objects corroborate this view. But some few have been led to contrary conclusions by observing the fact that in mitosis the nucleolus often disappears about the time that the centrosome becomes apparent. Thus Karsten ('93) assumes that the nucleoli wander out of the nucleus into the cytoplasm, and there become the centrosomes of the spindle ; this observation has been refuted by Humphrey ('94). Also Wasielevsky ('93) believes that the centrosomes of the egg of *Ascaris* stand in some connection to the nucleoli, but this stands in direct opposition to the conclusions of all other workers on this object, except those of Carnoy and Lebrun ('97b), and the supposition of Sala ('95). Then Lavdowsky ('94) concludes that the nucleolinus is the centrosome in the process of formation, but he failed to observe the steps by which this body develops into a centrosome. Further, Julin ('93b) is said by Delage ('95) to have assumed a genetic relation between the centrosome and the nucleolus. Other supporters of the nucleolar origin of the centrosome : Balbiani ('95), Wilcox ('95), Bremer ('95b), F. Toyama ('94). I believe that these are the only investigators who have assumed this genetic relation. We may conclude, from the greater number of observations at hand, that there is probably no connection between these structures in the metazoan cell. But it is difficult to decide the homologies of the body found by Keuten ('95) in the nucleus of *Ceratium*, and termed by him nucleolo-centrosoma ; he considers it as equivalent to the central

spindle and centrosome of *Ascaris*, but might it not be compared to the nucleolus alone, or to the nucleolus plus centrosomes of the metazoan cell? However, the significance of most protozoan "nucleoli" is very problematical. (*Cf.* the later observations of Lauterborn, '95a.)

### 9. *Ontogenetic Origin of the Nucleolus.*

Very few observations have been made to determine the mode of origin of the nucleolus, though there are numerous hypotheses intended to explain it. We may leave aside, for the time being, its mode of reappearance in the daughter-nuclei after nuclear division, since a special section will be devoted to that subject.

In order to determine the mode of origin of the nucleolus in resting stages of nuclei, I have studied those cells in which at first no nucleolus is present, but which after a certain period of growth acquire one. Objects well adapted for such investigation are the ova of the nemerteans and the mesenchym cells of *Cerebratulus*. For details of these processes the reader is referred to the observations.

In the ova of the nemerteans the nucleoli at the time of their first appearance are always in close contact with the nuclear membrane; this is also the case for the mesenchym cells of *Cerebratulus*, and probably for the paranucleoli of the ova of *Rodalia*. In all these cells the nucleoli only then leave the periphery of the nucleus and wander towards its center, after the nucleus has increased more or less in size. There is only one explanation for the peripheral position of the nucleoli at the time of their first appearance, namely, that their substance is extranuclear in origin. This process of formation has already been discussed in detail for the several cells, and it is not necessary to repeat here all the detailed observations on which the main deduction is based. If the nucleolar substance were a secretion of the nucleus, as Häcker ('95) assumes, how would this assumption explain the strictly peripheral position of the nucleoli when they first arise? For on Häcker's hypothesis we should expect the supposed nucleolar secretions to be de-

posited evenly throughout the nucleus, and not only at the periphery. And his deductions are based in great part, as those of most other investigators, on the study of maturation mitoses, and he had not observed their first mode of origin, namely, their origin in nuclei which are not in the prophases of mitosis, but are only gradually becoming differentiated from somatic cells. I have found no evidences in any cell that the nucleoli stand in any genetic relation to the chromatin elements of the nucleus ; and while the chromatin may derive substances from the nucleoli, I am unacquainted with any observations which show that the nucleoli derive any part of their substance from the chromatin. In all the cases observed by me, the nucleus appears to assimilate a substance or substances from the cytoplasm, and after this substance has entered the nucleus it apparently undergoes there a chemical change, and becomes deposited on the inner surface of the nuclear membrane in the form of masses of varying dimensions, which may be either globular or irregular in shape, according as they are fluid or viscid in consistency. In the case of the ova of the nemerteans the substance taken up into the nucleus, and which there becomes deposited in the form of nucleoli, is sometimes exactly similar to the substance of the yolk-balls which lie in the cytoplasm; in other cases it is probably similar to those metabolically changed portions or inclusions of the cytoplasm, out of which the yolk-balls are later differentiated. In *Lineus*, indeed, the yolk-balls may often be found halfway through the nuclear membrane, and their appearance is exactly similar to that of the nucleoli. In the mesenchym cells of *Cerebratulus* the substance of the nucleoli appears to be identical with that of the numerous nutritive granules which are dispersed in the cytoplasm ; the latter globules arise in the cytoplasm before the nucleolus appears in the nucleus, and as soon as they become numerous in the neighborhood of the nucleus, peripheral nucleoli begin to appear in the latter. In the subcutical gland cells of *Piscicola* the nucleolus, at the time of its most rapid growth, is apposed to the nuclear membrane; but when this period of volume-increase has ceased, it is never found in this position. Further, the paranucleoli of *Rodalia* appear first in contact with the

nuclear membrane. Schwalbe ('76) found in the nuclei of various vertebrate embryos that when the nucleoli first arise they appear as thickenings of the inner surface of the nuclear membrane.

From these observations I conclude, accordingly, that the nucleolar substance, in many if not all cells, has an extranuclear origin ; and that, though it may undergo a chemical change after entering the nucleus, it can be regarded neither as a secretion nor as an excretion of the latter. In making this conclusion I can corroborate the views of only one investigator, namely, Korschelt ('89), though he changed this opinion in a later paper ('97). He concluded that the nucleolar substance stands in some connection with the nutritive processes of the cell, and that the nucleus probably derives it from the cytoplasm.

Other views on the origin of the nucleolus (those of Häcker have already been mentioned): Auerbach ('74a, '76) first supposed the nucleolus to be cytoplasmic in origin ; more recently ('90) he appears to champion its nuclear origin. Rhumbler ('93) assumes that the "Binnenkörper" of *Protozoa* are products of the nucleus, but he does not attempt to decide whether those of the *Metazoa* have a similar origin. Strasburger ('82b) also postulates a nuclear origin for the nucleolus, and assumes that its substance is allied to chromatin. Jordan ('93) holds that the nucleoli probably arise from the chromatin threads. Flemming ('82) considers them to be "specifische Produkte des Kernstoffwechsels." Schwalbe ('76) supposes the nucleolar substance to be at first identical with that of the nuclear membrane, since he found it to arise as thickenings of the latter. C. Schneider ('91) supposes it to be a metamorphosed portion of the chromatin. Leydig ('83) concludes that the nucleoli are portions of the chromatin reticulum. Guignard ('85) assumes that they are derivatives of the chromatin filaments. Watasé ('94) considers them to be metabolic products of the cell, but he gives no detailed observations in regard to their mode of formation. Mertens ('93) and Retzius ('81) consider them to arise by concentration of the chromatin reticulum.

10. *Discharge of Nucleolar Substance from Resting Nuclei.*

Will ('84) holds that the larger nucleoli of the amphibian germinal vesicle pass out into the cytoplasm, and there become the yolk-nuclei ; and Scharff ('88) corroborates this view for the ova of *Trigla*, though it is opposed by Cunningham ('95). Macallum ('91) concludes that in amphibian ova the peripheral nucleoli generate a substance which diffuses first in the nucleus and from there into the cytoplasm, and that this substance combines with the cytoplasm to form the yolk substance ; Jordan ('93) expresses a somewhat similar view in regard to the yolk formation of the newt. Henneguy ('93) assumes that the corpuscle of Balbiani in the ova of *Vertebrata* "est très probablement une partie de la tache germinative, ou une tache germinative entiere, qui sort de la vésicule [germinative] pour penetrer dans le vitellus," and Mertens ('93) holds a similar view. And for egg cells of *Tunicata*, Floderus ('96) confirms Roule's ('84) observations, that the "intravitelline Körper" are paranucleoli which have wandered into the cell body. *Cf.* also Bremer ('95a, b).

Leydig ('88) finds that in ova of *Geophilus*, *Stenobothrus*, *Rana*, and *Triton* particles of nucleolar substance penetrate into the cytoplasm. Lukjanow ('88) concludes that in the case of the cells of the stomach mucosa of *Salamandra*, the nucleolus discharges a portion of its substance from the nucleus. Humphrey ('94), from observations on plant cells, maintains that in some cases portions of nucleolar substance may pass into the cytoplasm.

Fol ('83a, b) concludes that the follicle cells of the ascidian egg arise as buds from the surface of the germinal vesicle, and that each of these buds contains a particle of nucleolar substance ; these conclusions are affirmed by Roule ('83). Scharff ('88) supposes that the follicle cells of the ovum of *Gadus* are derived from nucleoli which have left the germinal vesicle, such nucleoli becoming the nuclei of the new cells. (Ogata '83) studied human pancreas cells and finds that a nucleolus wanders out of the nucleus, becomes a "Nebenkern," and the latter finally changes into the nucleus of a new cell, a conclusion which is opposed by Platner ('89b).

I have found a wandering of nucleolar substance out of resting nuclei in one very beautiful and unique case, namely, in the subcuticular gland cells of *Piscicola;* at one stage in its cycle of development the nucleus commences to contract in volume, and in so doing discharges all except a single one of its nucleoli into the cytoplasm. This and certain of the observations cited from other investigators show that a discharge of nucleolar substance from the resting nucleus takes place in some cells. But the more recent observations of Morgan, Floderus, and others on Tunicate development render it very probable that Fol and Roule were mistaken in assuming that the nucleoli which pass out of the germinal vesicle become the constituents of follicle cells. There is still some question, also, as to whether the nucleolar substance in the cytoplasm takes any part in the formation of the yolk substance. Other pertinent observations: Mertens ('93), Bremer ('95a, b), Kosinski ('87, '93), Galeotti ('95), Melissinos and Nicolaides ('90), Auerbach ('74), Ver Ecke ('93), Steinhaus ('88), Rohde ('96).

### 11. *Behavior of Nucleoli during Nuclear Division.*

It is in cases of nuclear division that the nucleolus has received the most attention from morphologists. The behavior of the nucleolus in mitosis and amitosis may be treated separately.

1. *Amitosis.* — In this mode of nuclear division it is frequently the case for the nucleolus to divide first, so that each of the daughter-nuclei receives a half, or approximately a half (for the division of the nucleolus is not always into two equal parts), of the parent-nucleolus. In support of this deduction the following observations may be mentioned: Schaudinn ('94, *Amoeba crystalligera*); F. E. Shulze ('75, *A. polypodia*); Will ('85, ova of *Nepa, Notonecta*); Doflein ('96, degenerating ova of *Tubularia*); Carnoy ('85, ova of *Gryllotalpa, Lithobius, Geotrupes*); Korschelt ('95, intestinal cells of *Ophryotrocha*); my observations on the peritoneal cells of *Polydora;* Hoyer ('90, intestinal epithelium of *Rhabdonema*); Frenzel ('93b, hepatopancreas cells of *Astacus*); Platner ('89a, Malpighian tubes of *Dytiscus;* Wheeler

('89, follicle cells of *Blatta*); de Bruyne ('97, follicle cells of *Nepa*, *Periplaneta*, *Meconema*, *Aeschna*). E. B. Wilson ('96), in speaking of amitosis, states: "In many cases, however, no preliminary fission of the nucleolus occurs; and Remak's scheme must therefore be regarded as one of the rarest forms of cell division." But the list of cases which I have given shows that such cases of nucleolar division are frequent in amitosis, so that I conclude that a fission of the nucleolus, if not exactly typical for this mode of nuclear division, is nevertheless well represented and occurs here much more frequently than in mitosis. Dr. E. G. Conklin has demonstrated to me preparations of nucleolar division in follicle cells of *Gryllus*, which he has kindly allowed me to mention here.

2. *Mitosis.* — In karyokinesis the nucleolus may either not disappear, or, and this is the most usual case, it disappears before the spindle is formed. These two modes may be considered in turn.

(*a*) *The nucleolus does not disappear.* — In some few cases the nucleolus wanders out into the cytoplasm after the disappearance of the nuclear membrane and may remain there for some time without undergoing any change. Such cases have been described by Häcker ('92a, egg of *Aequorea*), Wheeler ('95, that of *Myzostoma*), H. V. Wilson ('94, ova of *Tedamione* and *Hircinia*), Tangl ('82, flower buds of *Hemerocallis*), Gjurasin ('93, *Peziza*), and Karsten ('93, sporangia of *Psilotum*). In all these cases the nucleolus ultimately disappears in the cytoplasm, though in *Aequorea* it may be observed still in the cell body of one of the blastomeres at the thirty-two cell stage, and the daughter-nuclei produce their own nucleoli. (Similar are the observations of Mead, '95; Häcker, '96, '97; Rosen, '95; Zimmermann, '96; Metzner, '94; Foot '94; Poirault and Raciborski, '96.)

In the other cases where the nucleolus does not disappear it remains within the nucleus. In some of these cases it appears to divide into two or more parts; in other cases it may be that one of the daughter-nuclei receives the whole parent-nucleolus, while in the other one a new nucleolus is produced. There are a few observations which show that it sometimes divides; thus Strasburger ('82b, embryo sac of *Galanthus*) and Rosen ('92b,

*Synchytrium*) ; Reinke studied the mitosis of the spleen cells of the mouse, and found that the single parent-nucleolus divides into three or four pieces, while at the end of the mitosis each daughter-nucleus contains a single nucleolus. In the mitoses of the ovogonia of *Lineus* and *Polydora* my own observations show that the nucleolus persists in the nucleus, and each daughter-nucleus contains one nucleolus, so that it is very probable that in these cases the parent-nucleolus divides into two, and each daughter-nucleus thereby receives a half of it ; but these mitoses were so small that I was unable to decide this point definitely. Rosen ('95) finds nucleolar division in root cells of *Phaseolus ;* J. Wagner ('96a) describes a similar division of a "nucleolus" in spermatocytes of Arachnids, though this case, like that described by Henking ('90), probably represents a chromatin nucleolus. This persistence of the nucleolus in the nucleus during mitosis must be considered atypical.

(*b*) *The nucleolus disappears during mitosis.* — This is the most usual mode of behavior of the nucleolus during mitosis. The nucleolus either gradually diminishes in size, and so finally vanishes, or else it first fragments into a number of smaller pieces, and then these disappear. The only cell which I had for the study of this phenomenon was the ovum of *Piscicola* during the formation of the first pole spindle. When this spindle is complete no trace of nucleolar substance is to be seen anywhere in the cell. In stages immediately antecedent to that of the spindle, numerous minute granules, as well as a smaller number of larger globules, are dispersed through the nuclear sap; all these stain with eosin, and I regard them as particles of nucleolar substance which had become separated from the nucleolus. Thus a dissolution of the nucleolar substance commences before the nuclear membrane has disappeared, and after this membrane has vanished it is probable that all the nucleolar substance must be dissolved by the action of the cytoplasm, or at least become dispersed through the latter, so that no remnant of it is to be found in the region of the spindle or of the chromosomes. During the process of dissolution of the nucleolar substance in the nuclear sap the chromatin elements stain red (with eosin), and this fact may be

explained either by the assumption that the nucleolar substance unites chemically with the chromatin, or that it simply penetrates into the meshes of the latter; since no nucleolar substance appears to be united with any of the twelve chromosomes we may conclude that it does not unite chemically with the chromatin, and therefore the chromosomes probably do not serve to carry it over into the daughter-nuclei. We may now briefly review the results of other observers on the mode of disappearance of the nucleolus during mitosis.

It is not necessary to discuss the earlier view of O. Hertwig, which he has since discarded, that "der Eikern der aus dem Keimbläschen frei gewordene oder ausgewanderte Keimfleck ist," nor yet the view of Kölliker. Kleinenberg ('72) believes that the germinal spot of *Hydra* dissolves during mitosis; Brauer ('91) finds that it breaks into fragments, of which a part seems to be dissolved in the cytoplasm, "ein Theil tritt unverändert nach dem Schwinden der Membran in das Eiprotoplasma über." Fick ('93, germinal spot of *Amblystoma*) finds that the nucleoli disappear at the time of the longitudinal splitting of the chromosomes; and Böhm ('88) reaches the same conclusion for *Petromyzon*. Davidoff ('89, ovum of *Distaplia*) concludes "dass aus dem Nucleolus ein Kern mit Kernnetz, mit einem Nucleolus und Nucleolinus hervorgegangen ist"; and Vejdovský ('88, *Rhynchelmis*), Blochmann ('82, *Neritina*), and Marshall ('92, *Gregarina*) conclude that the nucleoli become chromosomes. In the egg of *Ascaris* the nucleoli gradually disappear, according to most observers. Strasburger ('82b) first contended that the nucleolar substance is taken up into the nuclear filaments; later ('88) he writes: "Auf Grund meiner neueren Erfahrungen erscheint es mir überhaupt unwahrscheinlich, dass die Nucleolarsubstanz, auch nach ihrer Auflösung im Kernsafte, den Kernfäden als Nahrung dienen sollte," and he considers that after it is dissolved in the nuclear sap a portion of it forms the cell membranes of the daughter-cells (*cf.* also his paper of '93). Rein ('83, ova of *Lepus* and *Cavia*) finds that the nucleolus breaks into small fragments, which finally disappear in the substance of the nucleus. Pfitzner ('83, ectoderm cells of *Hydra*) terms the nucleolar substance "prochromatin," since

he finds that in mitosis it changes into chromatin. Rabl ('85, larval cells of amphibians) and O. Schultze ('87, ova of *Rana* and *Triton*) contend that the nucleolar substance takes some part in the formation of the nuclear filaments ; but Born ('94) subsequently found that these filaments stand in no connection with the nucleolar substance. Holl ('93, ovum of *Mus*) finds that the central granules of the nucleoli wander out of them and so become the chromosomes. Van Beneden ('75, ovum of *Lepus*) originally supposed that the nucleolus becomes the first pole body. Kastschenko ('90, ova of *Selachii*) finds that all the nucleoli disappear in the spirem stage, while Rückert ('92) finds that a few of them pass into the cytoplasm. Stuhlmann ('86, ova of *Insecta*) finds that the nucleoli gradually disappear during the maturation of the egg ; and similar conclusions were reached by Stauffacher ('93, *Cyclas*), Rhumbler ('95, *Cyphoderia*), Sheldon ('90, *Peripatus*), Heathcote ('86, *Julus*), Van der Stricht ('95, *Amphioxus*), Brauer ('92, *Branchipus*), and Vejdovský ('82, *Sternaspis*). Auerbach ('96, spermatogonium of *Paludina*) holds that the nucleolar substance becomes incorporated with the chromatin elements. Meunier ('86) and Moll ('93) for *Spirogyra*, and Carnoy ('85) for other cells also, hold that the chromosomes are derivatives of the chromatin skein of the nucleolus. Heuser ('84, mitoses of various plant cells) contends that the nucleoli become gradually apposed to the nuclear filaments, and that their substance unites with these elements, though in some cases a superfluous portion of the nucleolar substance may be discharged from the nucleus. Korschelt ('95, ovum of *Ophryotrocha*) finds that the nucleolus gradually disappears by dissolving in the nuclear sap, and believes that a part of this substance may be introduced into the nuclear filaments. Zacharias ('85) somewhat prematurely concludes that the nucleoli always disappear in mitosis. Tangl ('82) finds that in *Hemerocallis*, in uninucleolar nuclei, the nucleolus dissolves in the nucleus, but in those which are multinucleolar one may pass out into the cytoplasm ; in *Hesperus* and *Cisium* they gradually disappear. Humphrey ('94, plant cells) holds that "die Nucleolen in einigen Fällen aus der Kernhöhle, bevor sie von den karyokinetischen Kräften angegriffen werden, austreten können."

Wager ('93, *Agaricus*) describes the nucleoli as becoming dissolved in the caryolymph, and then, this dissolved substance penetrating the chromatin elements, the latter serve to carry it over into the daughter-nuclei. Went ('87, plant cells) holds "dass in vielen Fällen wenigstens der Nucleolus beim Anfang der Kerntheilung im Kernfaden aufgenommen wird," and that "er sich nach der Theilung auch wieder daraus bildet." Rückert ('94, egg of *Cyclops*) finds that the nucleoli gradually break into fragments and the latter disappear. But there is not space here to mention all the views of students of mitosis.

There are only a few observations which would show that in mitosis the chromosomes are derived from the nucleoli (Davidoff, Vejdovský, Blochmann, Marshall, Sobotta, '95, Macallum, '95, Carnoy, '97a, R. Hertwig, '96, not corroborated by Brauer, '94), and these cases stand in such marked contradiction to the observations of other morphologists that a reinvestigation of them is very necessary.[1] Then we have the observations of Carnoy, Meunier, and Moll, which would show that the chromosomes are derived from a part of the nucleolus; but the existence of a "nucléole-noyau," *i.e.*, of a nucleus within a nucleus, as assumed by Carnoy and his followers, in any metazoan cell, seems to be very problematical. On the other hand, most observers agree that the nucleoli disappear more or less gradually during mitosis, and that the chromosomes are not derived from them. Now we have reached the crucial question: What is the mode of transference of the nucleolar substance to the daughter-nuclei? In answer to this, some observers hold that this substance may be distributed in the cytoplasm and taken up therefrom into the daughter-nuclei; others, that it combines with the chromatin elements and is transferred with these; still others maintain a position intermediate between these two.[2] But when we find so much variance in the conclusions of competent investigators only one deduction is allowable, namely, that the mode of transportation

[1] On the relation of nucleoli to chromosomes, *cf.* also Cunningham ('97), Sobotta ('95), Macallum ('95), Platner ('89c), Carnoy ('97a), R. Hertwig ('96), Van Beneden ('83), Zimmermann ('96), Lauterborn ('96), Boveri ('88), Wheeler ('97).

[2] *Cf.* also Belajeff ('94), Mottier ('97), and Rosen ('95).

of the nucleolar substance is probably different in different objects.

We have found above that in the simplest though secondary nuclear divisions, the amitotic, the nucleolar substance of the parent-cell is transported into the daughter-nuclei by the mechanically simplest process, namely, by a direct division of the parent-nucleolus ; this is very frequently the case in amitosis, though it does not always occur. But in most mitotic divisions the nucleolus first disappears, *i.e.*, there would seem to be an indirect mode of transference of its substance corresponding to the indirect mode of transference of the chromatin and linin elements. Now all mitotic divisions do not proceed on exactly the same plan, for we find differences in regard to the presence of a central spindle, in regard to the number of the chromosomes, etc. Accordingly, one would expect also different modes of transference of the nucleolar substances. Thus in some cases, as Wager ('93) suggests, the chromosomes may serve as mechanical vehicles for the transportation of this substance. In many other cases it is very probable that this substance, after the disappearance of the nuclear membrane, becomes dispersed in the cytoplasm ; and then each of the daughter-nuclei may either take up this substance from the cytoplasm again, or may produce its own nucleolus from a new substance, owing to the primitive nucleolar substance having been assimilated by, or even discharged from, the cytoplasm. There are observations in support of each of these three modes of re-formation of nucleoli in the daughter-nuclei. But since when the nuclear membrane disappears the cytoplasm probably comes into contact with the substance of the nucleoli, it is most probable that it would produce either a physical or a chemical change in the latter, and hence the second and third modes would appear the more probable. Accordingly, I agree with Humphrey ('94) that there is no substantial basis for Zimmermann's ('93) conclusion "omnis nucleolus e nucleolo," or more strictly speaking, that the nucleolus in most cases is not derived from a previously existing one. But the third mode of diffusion of the nucleolar substance is in reality not a transference of this substance at all, since it probably becomes lost in the cytoplasm ; and hence,

though the mode of disappearance of this substance may be more or less dependent upon the mode of mitosis, the substance of the parent-nucleolus may be in many cases not transferred to the daughter-nuclei, but the latter (perhaps as a rule) may produce their own nucleoli *de novo*.

Strasburger ('93, '97) assumes that the small granules found by Kostanecki ('92) in the equatorial plate of the central spindle may be nucleolar particles, and accordingly that the nucleolar substance may be in this way very evenly distributed to the daughter-nuclei; but it is not as yet clearly shown that these granules are derivatives of the nucleolus (*cf.* also Debski, '97, Sala, '95, Pfitzner, '86b, and Rosen, '95).

Zacharias ('85), Carnoy ('85), and Platner ('86) have concluded that in some cases the achromatic spindle fibers are derived from the nucleolus; similar views are held by Strasburger ('95, '97), Harper ('97), and Fairchild ('97), but most facts would show this view untenable.

Rhumbler ('93) assumes that a greater amount of nucleolar substance is accumulated in the nucleus before mitosis than is necessary for its growth, and this superfluous amount would serve for the formation of the nucleoli in the daughter-nuclei.

### 12. *The Function of the Nucleolus.*

The attempt to deduce the physiological economy of a structure from a mere study of its morphological relations is always difficult, and this is especially the case with regard to the nucleoli.

Balbiani ('64) found contractile and discharging vacuoles in the germinal spot of *Phalangium*, and notes that they differ from the contractile vacuoles of the *Rhizopoda* in that they are not formed again at the same point. Häcker ('93c) regarded the nucleolus of the ovum of *Echinus* as an excretory organ, since he found its large vacuole to be contractile; he compared it directly to the contractile vacuole of *Infusoria*. Balbiani ('65b) also observed contractile vacuoles in the germinal spots of *Helix*, *Vortex*, and *Prostomum*, and in these the vacuole discharges through a small orifice in the cortical substance of the

nucleolus. Böhm described ('88) the vacuole of the germinal spot of *Petromyzon* as connected by a fine duct with the surface of the nucleolus. Lukjanow ('88) found in the stomach cells of the salamander that the nucleolus is apposed to the nuclear membrane, through which it discharges an excretion. Compare also Van Bambeke ('97a) and Michel ('96). These observations would show that the nucleolus in some cases contains a contractile vacuole, and that the fluid substance of the latter is periodically discharged from it (*cf.* Hodge '94, Van Bambeke, '97, and Michel, '96).

Flemming ('82) considers the nucleoli to be nuclear organs, and regards them either as containers or reserve supplies of chromatin, or as "eine chemische Modification, Vorstufe oder Doppelverbindung" of the latter substance; this view is also held by Van Bambeke ('85). Zacharias ('85) also thinks that they are organs, but does not agree with Flemming that they are reserve masses of chromatin; Gjurasin ('93) corroborates the views of Zacharias. Strasburger originally contended ('84) that they represent reserve material, a view shared by many later observers; more recently ('88) he shows that the nucleolar substance may play some part in the formation of the cell membrane, but considers that they may also have some other, as yet unknown, function. Korschelt ('89) concludes that they are formed as depositions of nutritive substances, and that their substance "in und vielleicht ausserhalb des Kernes zur Verwendung gebracht werden sollte." Rhumbler ('93) assumes that the nucleoli ("Binnenkörper") of the *Protozoa* represent "Reservestoffe" deposited in the nucleus and consumed in the growth of the latter, standing in some connection with the chromatin; they are not organs, but secretions of the nucleus. Häcker ('95) concludes that they are not nuclear organs, but secretions of the nucleus formed in or from the chromatin elements and destined to be discharged from the nucleus during mitosis; he observes that the nucleolar substance "ein Stoffwechselsprodukt darstellt, dessen Erzeugung in einem gewissen Abhängigkeitsverhältniss zur Intensität der vegetativen Leistungen von Kern und Zelle steht," and that its amount stands in a direct ratio "zur Intensität der Wechselbeziehungen

zwischen Kern und Zelle"; he opposes the view "dass die Kernkörper aus dem Zellplasma in den Kern hineingelangen und hier in die Bildung des Chromatins eingehen." Leydig ('85) holds that certain of the nucleoli are differentiations of the chromatin reticulum, others of the "Kernplasma." Watasé ('94) considers that they may be metabolic products of the cell. Auerbach ('90) holds them to be the fundamental constituents of the nucleus, which is a retrogression to the earlier views of O. and R. Hertwig. Born ('94) states: "Die Nucleolen stehen in Beziehung zum individuellen Zellleben, nicht zur Fortpflanzung." Lavdowsky ('94) considers them to be reserve masses of chromatin. Macfarlane ('81, '85) regards them as the tropic centers of the cell, and as the most important mechanical agents in cell division. Julin ('96b) believes they conduct the vegetal processes of the cell. Mottier ('97) considers the nucleolus "ein Kraftvorrath, welcher der Zelle nach Bedarf zur Verfügung steht"; and Swingle ('97), as a reserve fund of nourishment for the kinoplasm in mitosis. Metzner ('94) considers them to be of importance in the processes of mitosis (compare his observations). Henneguy ('93) regards the nucleolus and Balbianian corpuscles as corresponding with the macronucleus of the *Infusoria* (*cf.* Julin, '93b). These, then, are the most important views on the nature of the nucleolus.[1]

From my own observations the nucleolar substance would seem to be extranuclear in origin, and not a secretion or excretion of the nucleus. To be sure it may, and probably does, undergo chemical changes within the nucleus, but it is derived in the first place from the cytoplasm. I regard the nucleoli as

[1] The following list includes, I believe, all who have written on the function of the nucleolus: Korschelt ('89), Häcker ('93a, '95, '97b), O. Hertwig ('77a, '92), Rhumbler ('93), R. Hertwig ('76, '96), Fick ('93), Lukjanow ('88), Brauer ('91), Nussbaum ('82), Strasburger ('82b, '84, '88, '95, '97), Jordan ('93), Flemming ('80, '82), Van Beneden ('75), Wasielevsky ('93), A. Schneider ('83), Henneguy ('93), Rückert ('92, '94), C. Schneider ('91), Born ('94), R. Wagner ('36, '37), Auerbach ('74a), Kölliker ('43), Lönnberg ('92), Klein ('78), Macallum ('91, '95), Stuhlmann ('86), O. Brandt ('78), Schwarz ('87), Giugnard ('85), Macfarlane ('81, '85, '92), Zacharias ('85), Watasé ('94), Humphrey ('94), Gjurasin ('93), Mann ('92), Julin ('93b), E. B. Wilson ('96), Van Bambeke ('85), Mottier ('97), Swingle ('97), Rosen ('95), Metzner ('94), Wheeler ('97), Carnoy ('84, '86, '97a, '97b).

consisting of a substance, or different substances, taken into the nucleus from the cell body. It seems probable, further, that these substances stand in some relation to the nutritive processes of the nucleus, and in a relation to the growth of the latter. Thus those nuclei which are characterized by an especially large amount of nucleolar substance are growing nuclei, *i.e.*, those of egg cells in the maturation period, those of the subcuticular gland cells of *Piscicola*, the mesenchym cells of *Cerebratulus*. In the gland cells of *Piscicola* the volume of the nucleolar substance rapidly increases in amount during the phase of growth of the nucleus, but diminishes when the latter decreases in volume. Somatic cells, on the contrary, at least those which are undergoing no dimensional changes, contain a relatively small amount of this substance. It is doubtful whether Häcker ('95) is quite correct in assuming that the amount of the nucleolar substance stands in a direct proportion to the intensity of the functional changes which take place between the nucleus and the cytoplasm; at least there are but few criteria to enable one to compute the degree of such an intensity. Thus one would suppose that in nerve cells there was a close and intimate correlation between nucleus and cell body, but the nucleoli of the ganglion cells of the nemerteans and *Piscicola* are very small. Häcker's deduction might be modified as follows: where there is a close physiological *rapport*, in regard to processes of nutrition, between the nucleus and the cell body a relatively large amount of nucleolar substance occurs in the former.

Accordingly, we find a relatively large amount of nucleolar substance in growing nuclei, and hence conclude that this substance stands in some connection with the processes of nutrition, is itself either nutritive in function or represents that portion of substances assimilated by the nucleus from which all nourishment has been extracted, and in this case it would be a waste product. A third possibility is that the nucleoli may represent accumulations of nutritive substance retained in the nucleus as a reserve supply; but this does not seem to be very probable, for by this assumption it would be difficult to explain the uniformity in the size of the nucleoli in a given species of cell.

It would be premature to attempt to decide the exact manner in which the nucleolar substance is concerned in the metabolism of the cell. But the facts at least show that it has an extranuclear origin, and is especially abundant in growing nuclei, which shows that it stands in intimate connection with the phenomena of nutrition of the nucleus.

Vacuoles are characteristic for certain stages in the development of many nucleoli, especially those of germinal vesicles. For the nucleoli of the ova of *Montagua* and *Doto*, I showed that the vacuolar substance is at first present in the form of small globules in the nuclear sap, that these become applied against the surface of the nucleolus, and, finally penetrating into the latter, represent within it the vacuoles. I was unable to decide the mode of derivation of the vacuoles for the other nucleoli studied. So in some cases this vacuolar substance would appear not to be a derivative of the ground substance of the nucleolus, but to be derived from without the latter. Thus such nucleoli may be considered as diosmosing structures. The manner of growth of nucleoli is apparently by a process of apposition of smaller particles of nucleolar substance to their surfaces, and the addition of vacuolar substance to them differs from this only in that the vacuolar substance is intussuscepted. This vacuolar substance may be also a product of the nutritive processes of the nucleus.

It is a difficult question to determine whether the nucleolus at some stage of its development should not be considered a nuclear organ. In most nuclei it has a regular shape, in others it may be oval; in many cases the nucleolus has no regular shape, and in the salivary gland cells of *Chironomus* (according to Balbiani) it is convoluted. From the facts at hand we may conclude that the shape of the nucleolus is pretty constant for the particular species of cell. Now, taking constancy in form as a criterion of an organ, one might conclude that the nucleoli are organs. But, on the other hand, the most frequent form of the nucleolus, namely, the spherical, might simply be due to its thin fluid consistency, and when it is more viscid in consistency its shape would be more irregular. Thus Rhumbler ('93) concludes that the irregular nucleoli of

*Foraminifera* "durch Zusammenfliessen anfänglich leicht flüssiger, dann zähflüssiger und schliesslich erstarrender Massen entstanden sind." It may be asked: Why does the nucleolus persist through the whole resting state of the nucleus if it be not an organ? It may be simply stored in the nucleus until at the time of mitosis, when the nuclear membrane disappears, it has an opportunity to leave the nucleus. The only observations which would prove that the nucleolar substance may functionate as an independent organ are those according to which the nucleolus contains a contractile vacuole, and thus rhythmically contract and expand; in these cases the nucleolus might be regarded as a pulsating excretory organ of the nucleus. The hypothesis might be suggested that though the nucleolus probably consists of substances which stand in some relation to the nutritive processes of the nucleus, and so at the time of its first formation may be a functionless, inert mass of substance, yet it may at later periods in the history of the resting nucleus acquire some active function and thus gradually come to acquire the value of a nuclear organ; this hypothesis is put forward merely as a tentative one. According to this view the nucleolus might be considered as an organ which serves to accumulate in itself the waste products of the nucleus, thus serving as a reservoir for such substances; or it might be considered as an organ of excretion, to discharge waste products out of the nucleus: in either case the nucleolus would seem to stand in direct connection with the nutritive substances and forces of the nucleus.

### 13. *Comparison of the Nucleoli in Plants, Protozoa, and Metazoa.*

I have made no morphological studies on the nucleoli of plant cells, but would judge from the results of botanical investigators that they are probably strictly comparable to the nucleoli of the metazoan cells.

Rhumbler ('93) doubts whether the nucleoli of the *Metazoa* and the "Binnenkörper" of the *Protozoa* are homologous structures; and, indeed, there are certain nucleolar structures

in *Protozoa* which are unique, such as the nucleolo-centrosome of Keuten ('95). Henneguy considers that the corpuscle of Balbiani, together with the nucleolar elements of the metazoan cell, corresponds to the macronucleus of the *Infusoria;* in connection with this view may be mentioned the observations of Bütschli ('80), according to which only the macronuclei of the *Ciliata* contain nucleoli. Henneguy's hypothesis is very ingenious, and opens an interesting field for investigation, but it is difficult to determine whether it corresponds to the facts at hand, or whether it does not.[1] Some of the nucleoli of *Protozoa* are comparable to those of *Metazoa*, but it is doubtful whether all of them are.[2] Thus it may be the case in some of the gregarines that the chromatin (or its physiological equivalent) is localized in some or all of the nucleoli, and such structures could not be compared with the nucleoli of the metazoan cell.

As to the metazoan nucleoli, there is the question whether the nucleoli of egg cells and of somatic cells should be considered homologous. In my opinion this may be answered in the affirmative, since the nucleoli of both kinds of cells appear to be depositions of substances which are concerned in the nutritive processes of the nucleus. In making this conclusion I limit myself to the true nucleoli and do not consider those structures which have been erroneously termed nucleoli, but which in reality are portions of the chromatin reticulum of the nucleus. Numerous writers have considered the thickened nodal points of the nuclear network to be nucleoli, and here may be mentioned Leydig, Klein, Waldeyer, and others. The "cyanophilic" nucleoli of Auerbach ('90), the "pseudonucleoli" of Rosen ('92a), the "nucléoles nucléiniens" of Carnoy ('85), and the "Karyosomata" of Ogata ('83), Lukjanow ('87b), and Macallum ('91) are undoubtedly not nucleoli but portions of the nuclear reticulum. While the "erythrophilic" nucleoli of Auerbach, the "Eunucleoli" of Rosen, the "nucléoles

[1] On the genetic relation of nucleoli to Balbianian corpuscles (true yolk-nuclei), a relation which seems to me very doubtful, *cf.* Mertens ('93), Galeotti ('95), Melissinos and Nicolaides ('90), Weismann and Ishikawa ('89), Ver Ecke ('93), Steinhaus ('88), Henneguy ('93), Julin ('93b).

[2] For the central masses of chromatin found in many protozoan nuclei, Doflein ('98) proposes the term "chromatosphere."

plasmatiques" of Carnoy, and the "Plasmosomata" of the other observers correspond to true nucleoli in the sense in which this term should be used. The existence of Carnoy's "nucléoles mixtes" and "nucléoles-noyaux" in cells of *Metazoa* appears to be doubtful. List ('96) considers that the paranucleoli of the egg cells and the nucleoli of the somatic cells are homologous, but that the nucleolus proper of the ova is different from both; but the chemical differences which he finds between these kinds of nucleoli do not prove that they are morphologically distinct structures.

## Appendix to the Literature Reviews.

Siebold ('39) noticed "in den Eiern von Plumatella campanulata Lam. . . . ein deutliches Keimbläschen mit gedoppeltem Keimflecke."

Koelliker ('43) concludes: "Es bestände . . . das Ei aus einer primitiven Zelle, dem Keimbläschen, die sich um einen Kern, den Keimfleck, gebildet, und um die sich nachher Körner und eine secundäre Zelle, die Dotterhaut, gelegt hätte."

Auerbach ('74a) was the first to emphasize and prove clearly that the number of nucleoli is usually quite large, and that they are frequently irregular in form (before this time it was generally assumed that the usual number of nucleoli was one or two). The nucleus is filled with "Grundsubstanz" (the "Zellsaft" of Kölliker) and "Zwischenkörnchen"; the latter are distinguishable from the nucleoli by their smaller size and different refraction. He explains the clear zone around the nucleolus and the "Kernkörperchenkreis" of Eimer by the action of a repulsive force on the part of the nucleolus and of the nuclear membrane. He distinguishes several successive stages of the nucleus with regard to the number of the nucleoli: *enucleolar* nuclei, at an early embryonal stage; *paucinucleolar* nuclei, with one or two nucleoli; *plurinucleolar* nuclei, with two to four; and *multinucleolar*, with more than four. "Die Zahl der Kernkörperchen in einem Kerne beträgt 1–16, und in extremen Fällen selbst noch viel mehr, bis über 190. Und zwar ist nur eine kleine Minderheit aller Kerne durch den Gehalt von nur

einem oder zwei Nucleoli ausgezeichnet." He gives a large series of data on the number and size of nucleoli in embryonal and adult cells of vertebrates and *Musca*. The enucleolar condition is characteristic for embryonal cells; later a nucleolus makes its appearance in the center of the nucleus, though its substance is probably derived from the cytoplasm; new nucleoli are formed by successive divisions of the first one. In *Teleostii* the nuclei have fewer nucleoli than those of *Amphibia*, and those of *Reptilia* fewer than those of *Mammalia;* from which is concluded that the number increases in advancing phylogeny as in the ontogeny. "Je schneller und absolut bedeutender das Wachsthum der Zellen ist, desto mehr scheint auch die Tendenz zur Vervielfältigung der Kernkörperchen obzuwalten." The nuclei of the stomach mucosa of *Rana* are multinucleolar in summer and autumn, while after hibernation they contain only one to four nucleoli, which may be due to a process of fusion. The substance of nucleoli is similar to that of the cytoplasm in structure, capability of movements and of producing vacuoles; just as the nucleus is first formed as a vacuole in the cytoplasm, so in the substance of a nucleolus (which is cytoplasmic in origin) a vacuole is formed which has the same relation to the nucleolus as the nucleus has to the cell; "bei dieser Betrachtungsweise erscheint demnach der Zellkern als ein hohler Brutraum, bestimmt, eine junge Zellenbrut in sich zu entwickeln, die Nucleoli aber als wahrhaft endogen entstandene Tochterzellen." In higher animals all nucleoli do not become daughter-cells, but fulfill some new function; "und so werden wir auch die ursprüngliche Bedeutung der Nucleoli als Fortpflanzungszellen nicht für ganz unmöglich halten dürfen, wenn wir auch auf der anderen Seite nicht zweifeln können, dass sie in den meisten Kernen der höheren Organismen ganz andere Aufgaben zu erfüllen haben müssen."

Auerbach ('74b) studied in life the fecundation and cleavage of *Strongylus* and *Ascaris*. A short time after the appearance of the two copulation nuclei in the ovum, arise in each from one to five nucleoli; "wenn eine Mehrzahl sich einfindet, so kommen sie nicht alle gleichzeitig, sondern eines nach dem anderen, in Intervallen von einer halben bis zu einigen Minuten

zum Vorschein, und zwar in unregelmässigen, oft beträchtlichen Entfernungen von einander." When the nuclei wander towards one another the nucleoli move about, "indem sie innerhalb des Kernraums allerlei gerade, zickzackförmige, bogenförmige, Bahnen durchlaufen, mit einer vergleichsweise erheblichen Geschwindigkeit, so dass zuweilen in weniger als einer Minute Strecken von der Länge des Kern-Durchmessers zurückgelegt werden"; during these movements the nucleoli remain perfectly spherical. When the copulation nuclei are apposed the nucleoli in them suddenly disappear, and the mode of this disappearance was determined in one case, though it is exceedingly rapid; "das Kügelchen wurde allmählich blasser und etwas grösser und fuhr dann plötzlich auseinander, ein Wölkchen bildend, welches einen Augenblick darauf nicht mehr zu sehen war." The nucleoli reappear in the resting nuclei, and in the successive generations up to the eight-cell stage have the same cycle of changes, except that in each generation they are somewhat larger than in the preceding. These nucleoli are formed independently of one another. By the re-formation of the nuclear vacuole a number of cytoplasmic granules pass into the cavity of the nucleus, and there fuse to form the nucleoli.

Reinhard ('82, cited by Braem, '97) describes in the egg of *Plumatella* different stages of the nucleoli, which may be single, double, or even trilobular.

WISTAR INSTITUTE OF ANATOMY AND BIOLOGY,
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## EXPLANATION OF PLATES XXI–XXX.

All the figures have been drawn with the aid of the camera lucida, and represent sections of the structures delineated. Those parts of them which are colored represent as accurately as possible the stained preparations from which they were copied; in most of the figures only certain portions are colored, the other details being filled in with the pencil. In order to show the correct proportionate size of the various cells and nuclei the greater number of the figures have been made at a magnification afforded by the homogeneous immersion lens $\frac{1}{12}$ of Zeiss, with the ocular 4, and unless otherwise specified this may be understood to have been the magnification employed. The following abbreviations have been used in the figures:

| | |
|---|---|
| *C.* | cell. |
| *C. D.* | cell duct. |
| *Cen.* | centrosome. |
| *Chr.* | chromatin. |
| *Chr. F.* | chromatin filament. |
| *Chrom.* | chromosome. |
| *C. Mb.* | cell membrane. |
| *C. Sp.* | centrosphere. |
| *C. T. N.* | nucleus of connective tissue. |
| *C. T. S.* | connective tissue sheath of the ovarial acinus. |
| *Cut.* | cuticula. |
| *Cy. Pl.* | cytoplasm. |
| *d. C.* | degenerated cells (or cell substance). |
| *End. Pl.* | endoplasm. |
| *Gon. Mb.* | gonadal membrane. |
| *Iv. Mb.* | intravitelline membrane. |
| *N.* | nucleus. |
| *N. Bd.* | problematical nuclear body. |
| *N. Fib.* | nuclear fibers. |
| *N. Gr.* | nuclear granules. |
| *N. Mb.* | nuclear membrane. |
| *N. P.* | metamorphosed portion of nucleus. |
| *N. Sap.* | nuclear sap. |
| *Nut. Gl.* | nutritive globule. |
| *n.* | nucleolus. |
| *n. 2.* | nucleolus of the second generation. |
| *n. D.* | derivatives of the nucleolar substance. |
| *n. Gr.* | granules of degenerated nucleoli. |
| *n. Mb.* | nucleolar membrane. |
| *nn.* | nucleolinus. |
| *n. Sub.* | nucleolar ground substance. |
| *n. Vac.* | nucleolar vacuole. |
| *nx.* | nucleolar body of unknown origin. |
| *Ps. n.* | pseudonucleolus. |
| *Secr.* | secretion corpuscles. |
| *Sp.* | spores. |
| *Sp. F.* | spindle fibers. |
| *Vac.* | vacuole. |
| *Yk. Bl.* | yolk ball. |
| *Yk. Gl.* | yolk globule. |

## EXPLANATION OF PLATE XXI.

*Figs. 1–19: Gregarines from Lineus gesserensis.*

FIG. 1. Smallest individual found (hom. immers., oc. 2. Hermann's fluid; Del. haematoxylin, eosin).

FIG. 2. Outline of the largest individual. Obj. C., oc. 2.

FIG. 3. Nucleus (corros. sublimate; Del. haematoxylin, eosin).

FIG. 4. Portion of a longitudinal section, though an individual in which spores were present (as in 3).

FIG. 5. The smaller of the two nuclei of Fig. 1.

FIG. 6. The same gregarine drawn in Fig. 1, but with obj. C., oc. 2 to show its relative size to the one of Fig. 2.

FIGS. 7–9. Nuclei (alcohol. sublimate; Ehrlich-Biondi stain, 3¼ hrs.).

FIG. 10. Nucleus (Flemming's fluid; Del. haematoxylin, eosin).

FIG. 11. Idem (Flemming's fluid; Ehrlich-Biondi stain, 23½ hrs.).

FIGS. 12–16. Nuclei (Flemming's fluid; Del. haematoxylin, eosin).

FIGS. 17–19. Idem (sublimate with 2% acetic acid; aq. sol. methylen blue, 30 min.; aq. sol. brasilin, 2¼ hrs.).

*Figs. 20–35: Gregarines from Carinella annulata (fixation with alcohol. sol. sublimate).*

FIGS. 20 and 21. Outlines of two individuals. Obj. C., oc. 2.

FIGS. 22–25. Nuclei (Del. haematoxylin, 15 min., alum carmine, 6 hrs.).

FIG. 26. Nucleus (Ehrlich-Biondi stain, 3 hrs.).

FIGS. 27 and 28. Nuclei (Del. haematoxylin, eosin).

FIG. 29. Nucleus, only the outlines of the nucleoli drawn.

FIGS. 30–35. Nuclei (as in 26).

*Figs. 36–49: Nuclei of ganglion cells from the brain of Doto (Fig. 36, of the smallest type of cell; Figs. 37–42, of medium-sized cells; Figs. 43–49, of the colossal cells).*

FIG. 36 (Hermann's fluid, 1¼ hrs.; Lyons blue, 15 min.).

FIGS. 37 and 38 (Hermann's fluid, 1¼ hrs.; Ehrl. haematoxylin, 1½ hrs., eosin, 7 min.).

FIGS. 39 and 40 (alcohol. sol. sublimate; Ehrlich-Biondi stain, 3¼ hrs.).

FIGS. 41 and 42 (as in 37).

FIG. 43 (as in 39).

FIG. 44 (Hermann's fluid, 1¼ hrs.; safranin, 92 hrs., gentian violet, 1½ hrs., orange G., 2 min.).

FIG. 45 (as in 36).

FIGS. 46 and 47. Two sections of one nucleus (as in 37).

FIG. 48 (as in 37).

FIG. 49 (as in 39).

FIG. 50. Immature germinal vesicle of *Emys* (picric acid; Del. haematoxylin).

*Figs. 51–56: Nuclei from the muscle cells of the circular musculature of Lineus gesserensis.*

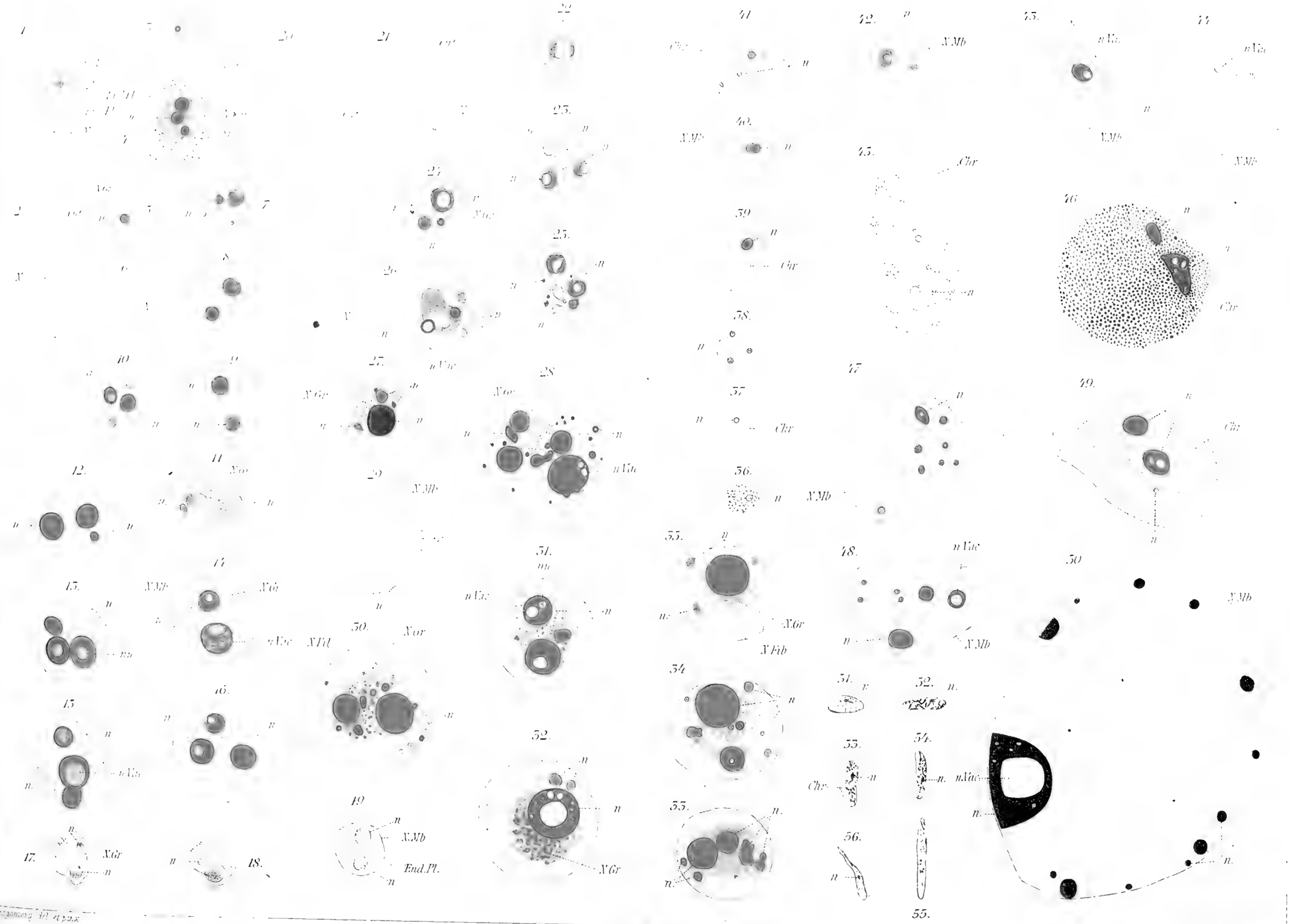
FIG. 51 (alcohol. sol. sublimate; Ehrlich-Biondi stain, 3¼ hrs.).

FIG. 52 (aq. sol. sublimate; cochineal, 1 hr., Del. haematoxylin, 20 min.).

FIGS. 53 and 54 (aq. sol. sublimate with 2% acetic acid; Ehrl. haematoxylin, eosin).

FIG. 55 (Hermann's fluid, 30 min.; Ehrl. haematoxylin, 3 hrs.; eosin, 5 min.).

FIG. 56 (as in 54).




Montgomery del. et lith.

Lith. Werner & Winter, Frankfurt a/M.

## EXPLANATION OF PLATE XXII.

*Figs. 57–63, 65–87: Germinal vesicles of Montagua pilata; Figs. 64, 88, 89: germinal vesicles of Doto.*

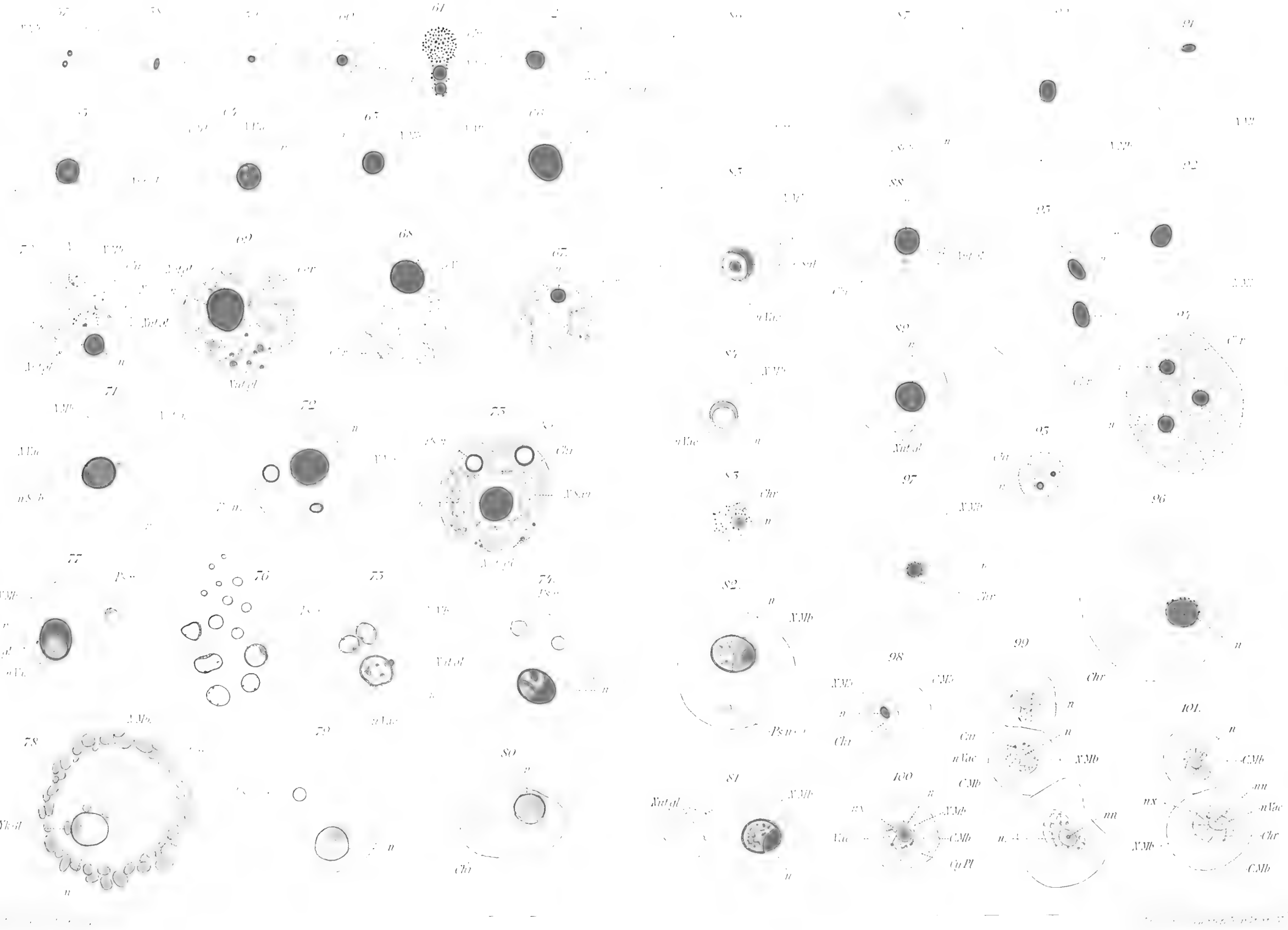
FIGS. 57–59 (alcohol. sol. sublimate; Ehrlich-Biondi stain, 3 hrs.).
FIG. 60 (alcohol. sol. sublimate; Ehrl. haematoxylin, 1 hr.; eosin, 5 min.).
FIGS. 61–63 (aq. sol. sublimate; Del. haematoxylin, 25 min.; eosin, 5 min.).
FIG. 64 (alcohol. sol. sublimate; Ehrlich-Biondi stain, 3¼ hrs.).
FIGS. 65–69 (as in 60).
FIG. 70 (as in 61).
FIG. 71 (as in 57).
FIGS. 72–75 (as in 61).
FIG. 76. Outlines of pseudonucleoli from various ova of one individual (aq. sol. sublimate).
FIG. 77 (as in 61).
FIGS. 78–80 (alcohol. sol. sublimate; Mayer's acid carmine, 15 min.; nigrosine, 10 min.).
FIGS. 81–87 (Flemming's fluid; Del. haematoxylin, eosin).
FIGS. 88 and 89 (as in 64).

*Figs. 90–97: Nuclei of ganglion cells from the brain of Montagua pilata (Fig. 93, from a cell of medium size; the others from the colossal cells).*

FIGS. 90 and 91 (alcohol. sol. sublimate; Ehrlich-Biondi stain, 3 hrs.).
FIGS. 92–94 (picrosulphuric acid; Del. haematoxylin, 25 min.; eosin, 5 min.).
FIG. 95 (as in 90).
FIGS. 96 and 97 (Flemming's fluid; Del. haematoxylin, eosin).

*Figs. 98–101: Blood corpuscles of Doto.*

FIG. 98 (alcohol. sol. sublimate; Ehrlich-Biondi stain, 3¼ hrs.).
FIGS. 99–101 (Hermann's fluid, 1¼ hrs.; safranin, 92 hrs.; gentian violet, 1½ hrs.; orange G., 2 min.).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## EXPLANATION OF PLATE XXIII.

FIG. 102. Blood corpuscle of *Doto* (picro-nitro-osmic acid, 35 min.; Del. haematoxylin, 30 min.; eosin, 5 min.).

*Figs. 103–133: Egg development of Tetrastemma catenulatum.*

FIGS. 103–106. Germinal vesicles (aq. sol. sublimate; Ranvier's picrocarmine; Del. haematoxylin).

FIG. 107. Portion of a young gonad (as in 103).

FIG. 108. Immature ovum (as in 103).

FIG. 109. Germinal vesicle (aq. sol. sublimate; Del. haematoxylin, 25 min.; eosin, 5 min.).

FIGS. 110 and 111. Germinal vesicles (aq. sol. sublimate; Del. haematoxylin, 15 min.; eosin, 5 min.).

FIG. 112. Germinal vesicle with portion of the surrounding cytoplasm (as in 110).

FIG. 113. Germinal vesicle (as in 110).

FIG. 114. Idem, with portion of the surrounding cytoplasm (as in 110).

FIGS. 115 and 116. Outlines of young ova (as in 110).

FIGS. 117–119. Germinal vesicles (as in 110).

FIG. 120. Outline of germinal vesicle, the natural color of the nucleoli shown (aq. sol. sublimate).

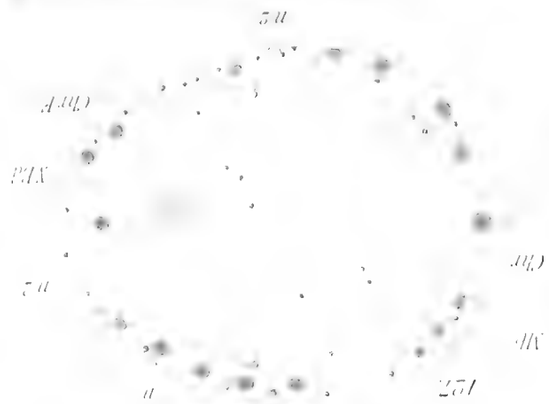
FIG. 121. Tangential section of the inner surface of the nuclear membrane, the dotted line representing the greatest diameter of the nucleus (as in 109).

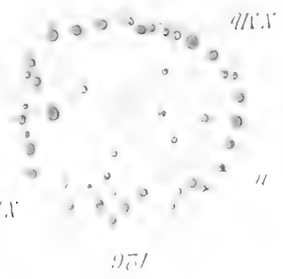
FIGS. 122–133. Germinal vesicles (as in 109).

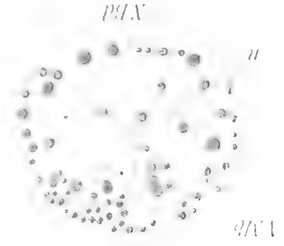
*Figs. 134–136: Outlines of the nuclei of ganglion cells of Piscicola.*

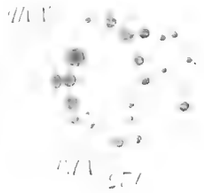
FIGS. 134 and 135 (alcohol. sol. sublimate).

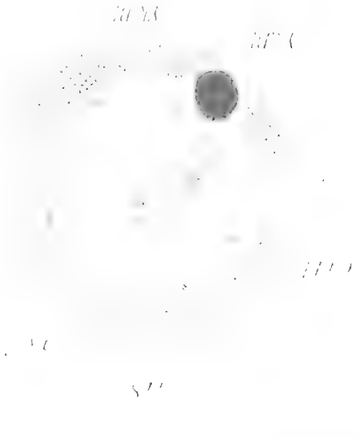
FIG. 136 (Flemming's fluid, 1 hr.).

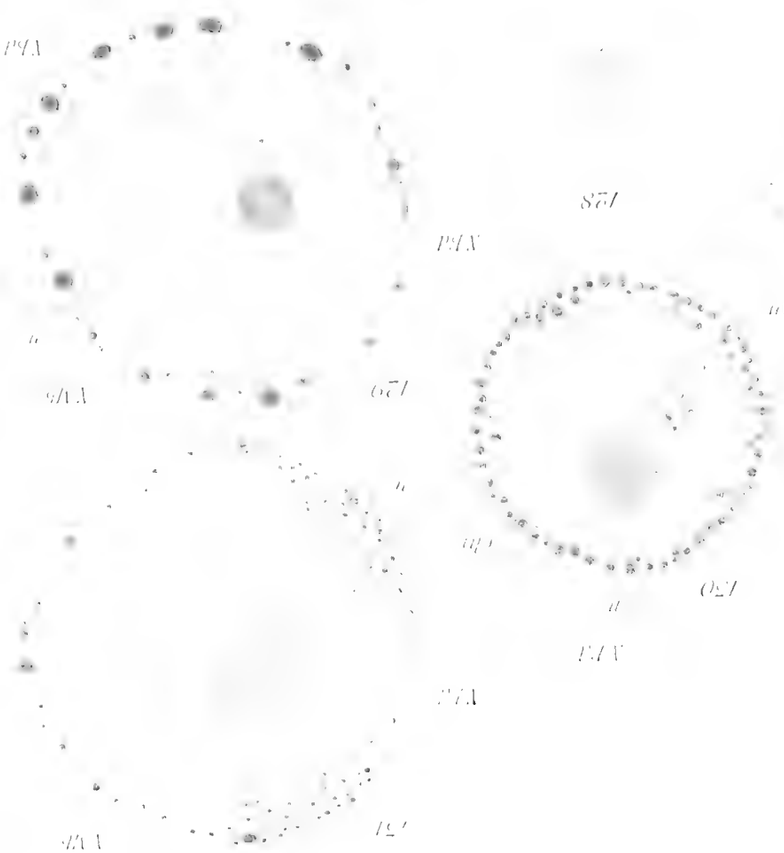












## EXPLANATION OF PLATE XXIV.

FIGS. 137–139. Germinal vesicles of *Tetrastemma catenulatum*, the nucleoli omitted in Fig. 139 (aq. sol. sublimate; Del. haematoxylin, 25 min.; eosin, 5 min.).

*Figs. 140–158: Egg development of Amphiporus glutinosus.*

FIGS. 140–143. Germinal vesicles (aq. sol. sublimate; Del. haematoxylin, 20 min.; eosin, 5 min.).

FIGS. 144–146. Germinal vesicles with surrounding cytoplasm (as in 143).

FIGS. 147–150. Germinal vesicles (as in 143).

FIG. 151. An abnormally large yolk ball (aq. sol. sublimate; aq. sol. dahlia, 15 min.; eosin, 5 min.).

FIGS. 152–154. Germinal vesicles (aq. sol. sublimate; haematoxylin, 45 min.; ferro-ammonio-sulphate, 45 min.; haematoxylin, 45 min.).

FIG. 155. Ovum and a part of the gonadal cavity in which yolk balls lie, only a portion of the cytoplasm drawn (as in 152).

FIGS. 156–158. Germinal vesicles (as in 154).

*Figs. 159–177: Egg development of Lineus gesserensis.*

FIG. 159. Nuclei from which the germinal vesicles are derived, from the cytoplasm of the gonad (Hermann's fluid; safranin, 70 hrs.; gentian violet, 1 hr.; orange G., 2 min.).

FIG. 160. Ovum (as in 159).

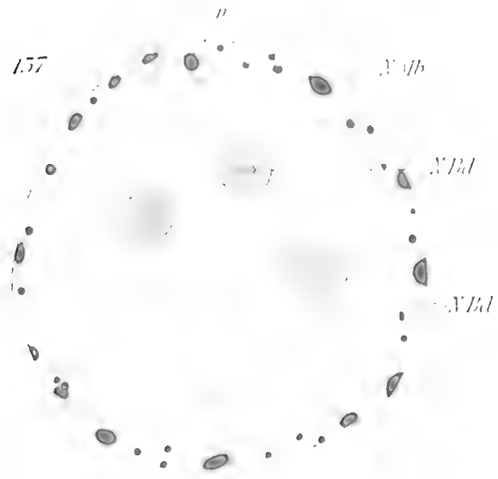
FIGS. 161 and 162. Germinal vesicles (as in 159).

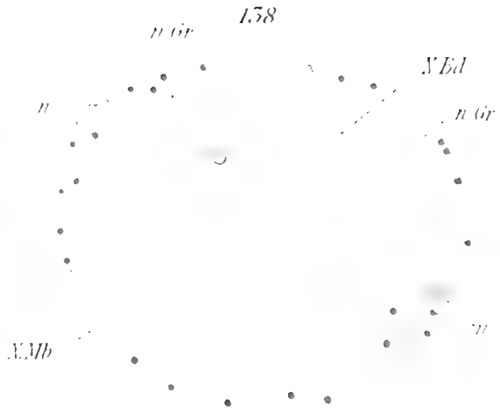
FIG. 163. Group of neighboring nuclei from a gonad, showing mitotic stages (aq. sol. sublimate; Del. haematoxylin, 20 min.; eosin, 5 min.).

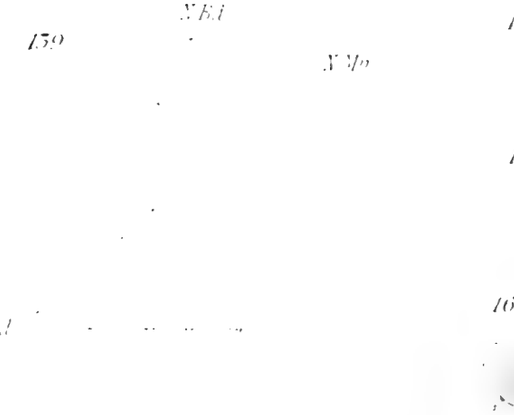
FIGS. 164–172. Nuclei from gonads (as in 163).

FIGS. 173–176. Germinal vesicles (as in 163).

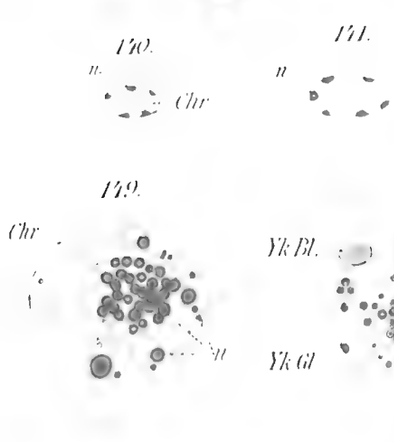
FIG. 177. The largest ovum found, only a part of the cytoplasm drawn (as in 163).


137.
n
X.Mb
X.Bd
X.Bd
X.Bd


138.
n.Gr
X.Bd
n.Gr
X.Mb
n

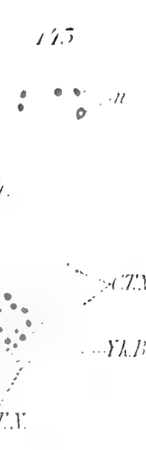

139.
X.Bd
X.Mb
X.Bd
n

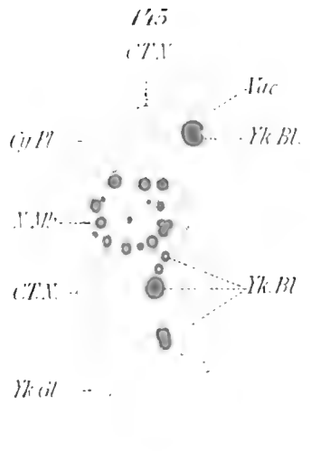

165.
Chr
166.
n
169.
n
Chr.F
170.
n
164.
n
167.
Chr.F
168.
Nut.gl
Chr
n
171.
ChrF
n
X.Mb
162.
n
Chr
163.
a
ChrF
b
c
X.Mb
d
j
i
h
ChrF
n
e
f
g
172.
ChrF
n.
X.Mb
n

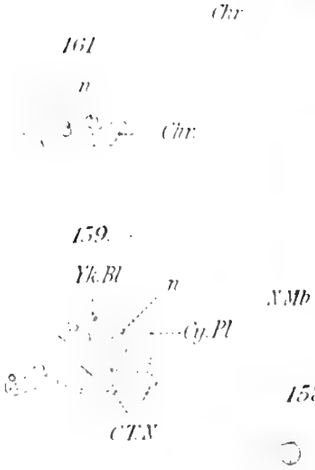

140.
n.
Chr


141.
n


142.
n


143
n


145
CTX
Xac
Yk.Bl.
Cy.Pl
X.Mb
Yk.Bl
CTX
Yk.Gl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n
Chr
160
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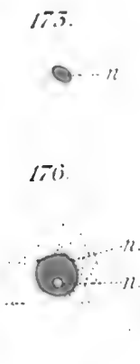
CMb
Yk.Bl.
X.Mb
n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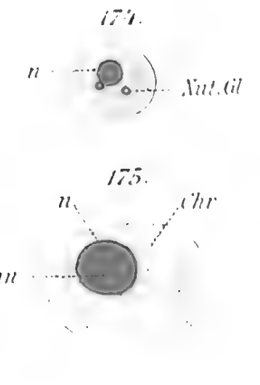
n


174.
n
Nut.Gl


149.
Chr
n


148.
n
Yk.Bl.
Yk.Gl
X.Mb

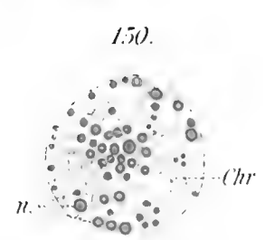

144.
Yk.Gl
n
Yk.Bl.
CTX
CTX
Yk.Bl
Cy.Pl
CTX

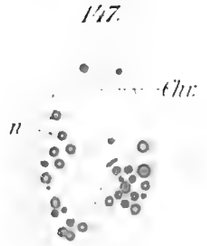

159.
Yk.Bl
n
Cy.Pl


158.
n.
n.Vac
X.Mb.


176.
n.
n.Vac.
Chr.


175.
n
Chr
nn


150.
Chr
n.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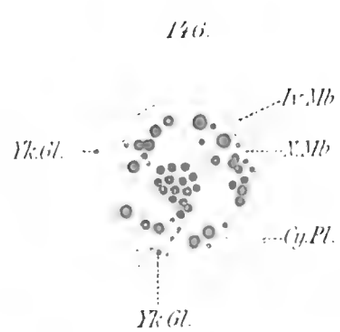
Chr.
n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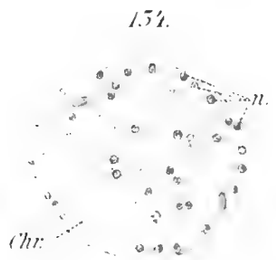
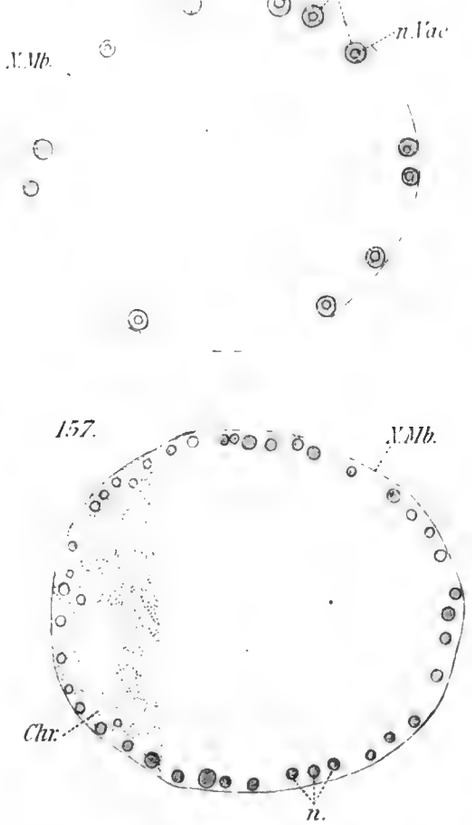
Iv.Mb
X.Mb
Yk.Gl.
Cy.Pl.
Yk.Gl.


154.
n.
Chr.


177.
N.Mb.
C.Mb
Cy.Pl.
n
Nut.gl
Nut.gl
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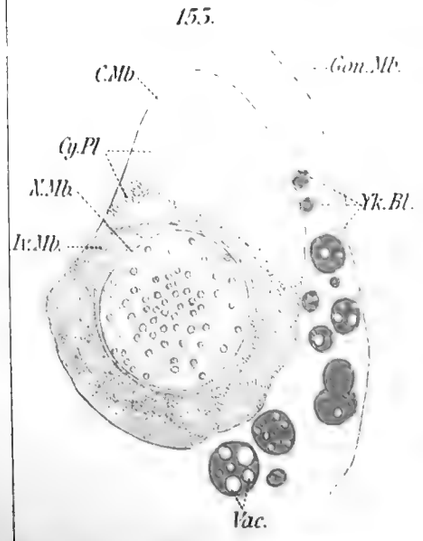
Yk.Bl.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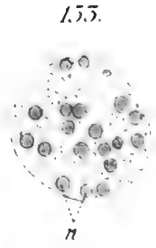
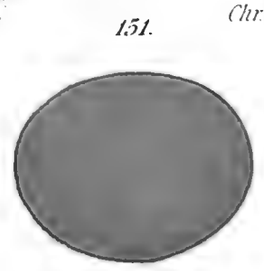
Cy.Pl
X.Mb
Yk.Bl.
Iv.Mb
Vac.


153.
n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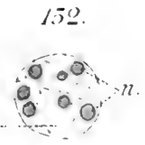
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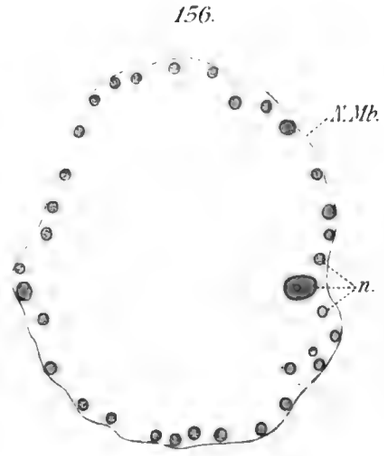
n.
Chr.


156.
X.Mb.
n.
n.


157.
X.Mb.
Chr.
n.

## EXPLANATION OF PLATE XXV.

*All the figures refer to the large subcuticular gland cells of Piscicola rapax Figs. 178–196 show stages of the prophase, and Fig. 167 the commencement of the metaphase of the nucleus.*

FIG. 178. Outline of an immature cell, only a portion of its duct drawn (aq. sol. sublimate).

FIGS. 179–181. Immature cells (aq. sol. sublimate; Mayer's acid carmine, 20 min.; nigrosine, 25 min.).

FIG. 182. Immature nucleus (alcohol. sol. sublimate).

FIG. 183. Idem (alcohol. sol. sublimate; Ehrlich-Biondi stain, 3 hrs.).

FIGS. 184–189. Nuclei (alcohol. sol. sublimate).

FIGS. 190–194. Stages of the ramification of the nucleus (Flemming's fluid).

FIGS. 195 and 196. Nuclei at the end of the prophase (alcohol. sol. sublimate).

FIG. 197. Nucleus at the commencement of the metaphase, discharging its nucleoli (Flemming's fluid).

178

179

180

181

182

183

184

185

186

187

188

189

190

191

192

193

194

195

196

197

Chr

Vac

XMb

n

cp Pl

n Vac

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## EXPLANATION OF PLATE XXVI.

*Figs. 198–203: Large subcuticular gland cells of Piscicola, in stages of the metaphase.*

FIGS. 198 and 199. Nuclei discharging their nucleoli, only outlines drawn (Flemming's fluid).

FIGS. 200–203. Subsequent stages of the metaphase (as in 198).

*Figs. 204–212: Egg development of a siphonophore (Rodalia (?); all fixed in alcohol and stained with Del. haematoxylin).*

FIG. 204. Ovum from gonophore, chromatin unstained. Obj. A, oc. 4.

FIG. 205. Ovum from egg pouch. Obj. C, oc. 4.

FIG. 206. Germinal vesicle from egg pouch. Obj. C, oc. 4.

FIGS. 207–209. Germinal vesicles from gonophores. Obj. C, oc. 4.

FIG. 210. Ovum from egg pouch. Obj. C, oc. 4.

FIG. 211. A large and a small ovum from an egg pouch. Obj. C, oc. 4.

FIG. 212. Nucleolus from a large ovum of a gonophore.

198.

n.

n

X.Mb

CMb

199.

Cy.Pl.

X.Mb

n

200.

X.Mb

n.

CMb

Cy.Pl.

201.

n.

X.Mb

n.D.

CMb.

Secr.

202.

Cy.Pl.

CD.

CMb.

n.D.

CMb

n.D.

X.

203.

n.D.

X.Mb

CMb

n

n.D.

Cy.Pl.

CD

204.

X.Mb

n

CMb

Cy.Pl.

205.

CMb.

Cy.Pl.

n.

Chr

X.Mb

206.

X.Mb

n.

n.Vac.

Chr.

207.

n.

n.2.

Chr.

208.

X.Mb

n

209.

n.

n.2.

Chr.

n.2.

X.Mb

210.

X.Mb

n

CMb

Cy.Pl.

X.Mb

n

211.

n.

X.Mb

CMb

Cy.Pl.

Gen.Mb

X.Mb

n.

n.

X.Mb

Cy.Pl.

212.

n.Vac.

n.Sub.

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## EXPLANATION OF PLATE XXVII.

*Figs. 213–235: Egg development of Stichostemma eilhardi.*

FIG. 213. Portion of the cell syncytium of an immature gonad (aq. sol. sublimate; aq. sol methylen blue, 5 min.; brasilin, 20 min.).

FIG. 214. Germinal vesicle (as in 213).

FIG. 215. Yolk balls (as in 213).

FIG. 216. Germinal vesicle (as in 213).

FIGS. 217 and 218. Portions of cell syncytia of gonads (as in 213).

FIG. 219. Germinal vesicle (aq. sol. sublimate; Del. haematoxylin, 15 min.; borax carmine, 20 hrs.).

FIG. 220. Portion of the cell syncytium of a gonad (aq. sol. sublimate; Ehrlich-Biondi stain, 3 hrs.).

FIGS. 221–223. Germinal vesicles (as in 220).

FIGS. 224–227. Idem (Flemming's fluid; alum carmine, 24 hrs.).

FIG. 228. Portion of a gonadal syncytium (aq. sol. sublimate; Del. haematoxylin, 15 min.; alum carmine, 22 hrs.).

FIGS. 229 and 230. Germinal vesicles (as in 228).

FIG. 231. Germinal vesicle (aq. sol. sublimate; Del. haematoxylin, 15 min.; alum carmine, 45 hrs.).

FIG. 232. Idem (aq. sol. sublimate; picrocarmine, 22 hrs.).

FIG. 233. Ovum, only a portion of the cytoplasm drawn (Lang's fluid; alum carmine; Del. haematoxylin, 15 min.).

FIG. 234. Germinal vesicle (aq. sol. sublimate; Del. haematoxylin, 15 min.; alum carmine, 16 hrs.).

FIG. 235. Germinal vesicle and portion of the cytoplasm (aq. sol. sublimate; Del. haematoxylin, 15 min.; alum carmine, 24 hrs.).

*Figs. 236–248: Egg development of Zygonemertes virescens.*

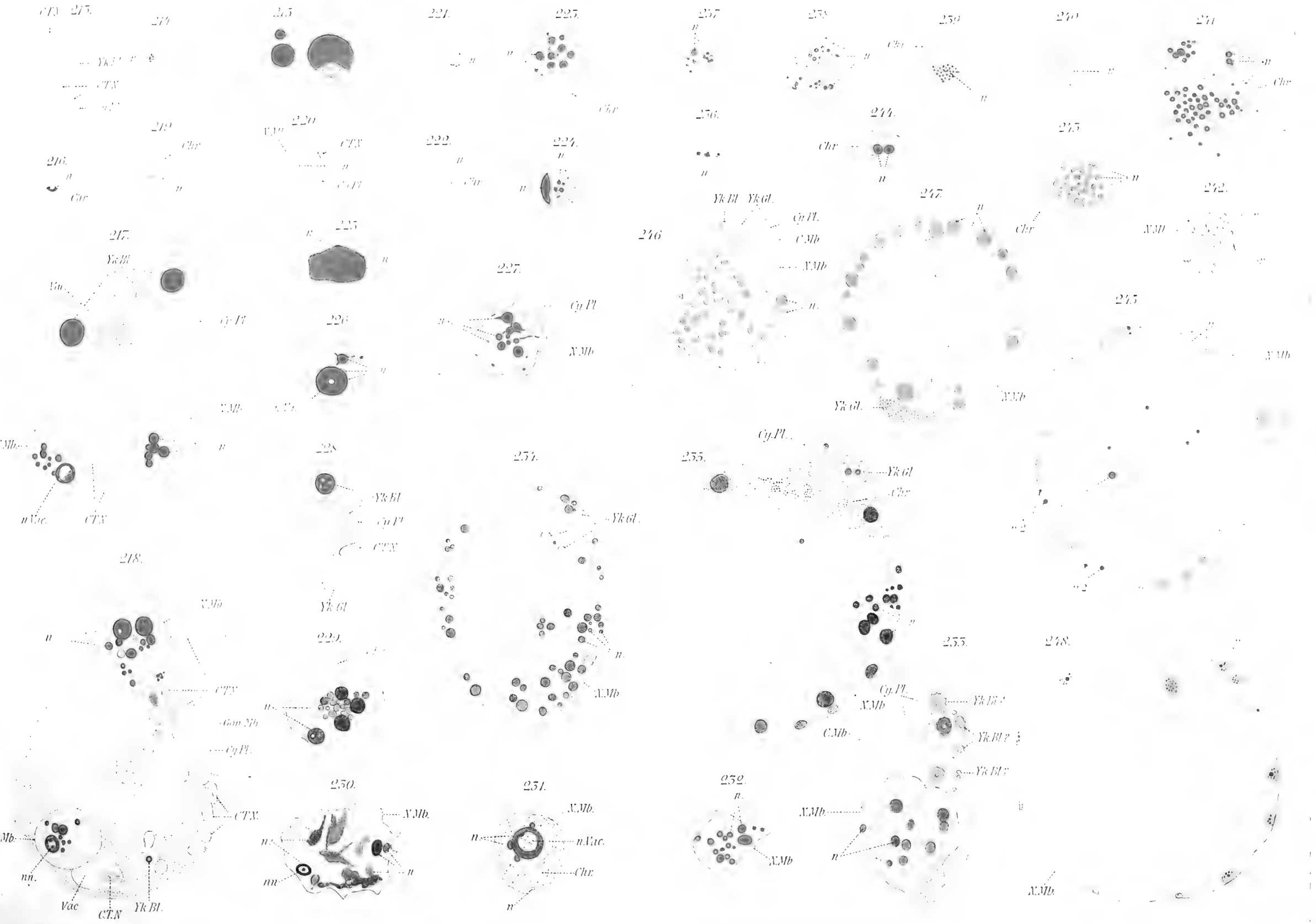
FIGS. 236–241. Germinal vesicles (aq. sol. sublimate; Del haematoxylin, 20 min.; eosin, 5 min.).

FIGS. 242 and 243. Idem (aq sol. sublimate; Ehrlich-Biondi stain, 3 hrs.).

FIGS. 244 and 245. Idem (alcohol. sol. sublimate; picrocarmine; Del. haematoxylin, 20 min.; eosin, 5 min.).

FIG. 246. Portion of an ovum (as in 242).

FIGS. 247 and 248. Germinal vesicles (as in 242).

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Lith. Werner & Winter Frankfurt a/M.

## EXPLANATION OF PLATE XXVIII.

*Figs. 249–281: Egg development of Polydora.*

FIG. 249. Nuclear division in a peritoneal cell (alcohol. sol. sublimate; Ehrl. haematoxylin, 1 hr.; eosin, 5 min.).

FIGS. 250–254. Free cells of the body cavity (as in 249).

FIG. 255. Nuclear mitosis (as in 249).

FIGS. 256–259. Mitoses of genital cells (as in 249).

FIG. 260. Nuclear mitosis (as in 249).

FIGS. 261–266. Immature ova (as in 249).

FIGS. 267 and 268. Germinal vesicles (as in 249).

FIG. 269. Ovum, only a portion of the cytoplasm drawn (as in 249).

FIGS. 270 and 271. Ova (aq. sol. sublimate with 5% acetic acid; Ehrlich-Biondi stain, 3 hrs.).

FIGS. 272–275. Germinal vesicles (as in 270).

FIGS. 276 and 277. Idem (Perenyi's fluid, 1 hr.; Ehrlich-Biondi stain, 2½ hrs.).

FIG. 278. Ovum, only a portion of the cytoplasm drawn (Flemming's fluid; safranin, 70 hrs.; gentian violet, 2¼ hrs.; orange G, 2 min.).

FIGS. 279–281. Germinal vesicles (as in 278).

*Figs. 282–299: Egg development of Tetrastemma elegans.*

FIGS. 282–291. Germinal vesicles (alcohol. sol. sublimate; Del. haematoxylin, 25 min.; eosin, 5 min.).

FIG. 292. Ova (Hermann's fluid; Del. haematoxylin, 45 min.; eosin, 5 min.).

FIGS. 293–299. Germinal vesicles (as in 292).

## EXPLANATION OF PLATE XXIX.

*Figs. 300–316: Egg development of Piscicola rapax.*

FIGS. 300–304. Transverse sections of ovarial acini (alcohol. sol. sublimate; Ehrl. haematoxylin, 1 hr.; eosin, 5 min.).

FIGS. 305 and 306. Germinal vesicles (as in 300).

FIG. 307. Ovum (as in 300).

FIG. 308. Germinal vesicle (as in 300).

FIGS. 309 and 310. Ova (as in 300).

FIG. 311. First pole spindle in the ovum; only one attraction sphere is drawn, and that only partially, the dotted line showing how far its rays extend into the cytoplasm (as in 300).

FIGS. 312 and 313. Germinal vesicles (alcohol. sol. sublimate; Mayer's acid carmine, 20 min.; Lyons blue, 5 min.).

FIG. 314. Germinal vesicle; the dotted line shows the extension of the indented surface of the nucleolus, the unstained small oval space being the external opening into it (alcohol. sol. sublimate; fuchsine, 10 min.).

FIG. 315. Germinal vesicle (Flemming's fluid; Ehrl. haematoxylin, 2 hrs.; eosin, 10 min.).

FIG. 316. Ovum with attraction spheres at opposite ends of the nucleus; the rays of only one attraction sphere drawn (alcohol. sol. sublimate; Ehrl. haematoxylin, 40 min.; eosin, 5½ min.).

*Figs. 315a–324: Mesenchym cells of Cerebratulus lacteus (fixation with alcohol. sol. sublimate).*

FIG. 315a. Nucleus (Ehrlich-Biondi stain, 2 hrs.).

FIGS. 316a and 317. Nuclear division in free cells (Ehrl. haematoxylin, 2 hrs.; eosin, 5 min.).

FIGS. 318 and 319. Nuclei (as in 317).

FIGS. 320–324. Whole cells (as in 317).

*Figs. 325–337: Nuclei of the muscle cells of the longitudinal musculature of Piscicola rapax.*

FIG. 325 (aq. sol. sublimate).

FIG. 326 (alcohol. sol. sublimate; Ehrl. haematoxylin, 1 hr.; eosin, 5 min.).

FIG. 327 (Flemming's fluid, 1 hr.).

FIG. 328 (aq. sol sublimate).

FIG. 329 (as in 327).

FIGS. 330 and 331 (as in 328).

FIG. 332 (as in 327).

FIG. 333 (Flemming's fluid, 1 hr.).

FIGS. 334 and 335 (as in 326).

FIGS. 336 and 337 (as in 333).


304.
305.
306.
307.
312.
313.
314.
315.
316.
317.
318.
319.
320.
321.
322.
323.
324.
325.
326.
327.
328.
329.
330.
331.
332.
333.
334.
335.
336.
337.
CIV
CIS
CMb
XMb
n
XSap.
ChrE
Chrom
SpE
Cen
n.Mac.
ChrO
XSap.
XMb
CSp.
n.D
Xut gl.
GyPl.

## EXPLANATION OF PLATE XXX.

*All figures refer to the giant cells of Doto.*

FIG. 338. Nucleus (alcohol. sol. sublimate; Ehrlich-Biondi stain, 3¼ hrs.).

FIG. 339. Cell (as in 338; hom. immers., oc. 2).

FIGS. 340 and 341. Nuclei (Hermann's fluid, 1¼ hrs.; safranin, 92 hrs.; gentian violet, 1½ hrs.; orange G., 2 min.).

FIGS. 342 and 343. Two sections of a single nucleus (as in 338).

FIG. 344. Nucleus (Hermann's fluid; Ehrl. haematoxylin, 1½ hrs.; eosin, 7 min.).

FIG. 345. Dividing nucleolus (as in 344).

FIG. 346. Nucleus (as in 344).

338

r

V.Mb

337

Chr

n

V.Mb

344.

n

V.Mb

n Vac.

n

342

n

Chr

V.Mb

343

n

343

n Vac.

n

V.Mb

341

n

n Vac.

V.Mb.

340.

n

Chr

V.Mb

n.Vac.

346.

V.Mb

n

n Vac

n

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# MORPHOLOGY.

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# STUDIES ON THE MATURATION, FERTILIZATION, AND CLEAVAGE OF THALASSEMA AND ZIRPHAEA.[1]

BRADNEY B. GRIFFIN.

CONTENTS.



[1] The death of Mr. Griffin, in April, 1898, occurred before the final revision of the manuscript of the following paper, which had been placed in my hands as a

## INTRODUCTION.

In the autumn of 1895, Prof. E. B. Wilson placed in my hands a very complete series of maturation and fertilization stages of the echiuroid *Thalassema mellita* Conn, collected by him at Beaufort, N. C., for a careful study of the achromatic structures with especial reference to the structure and function of the centrosome in maturation and fertilization. The research was at first exclusively confined to *Thalassema*, which proved an extremely favorable object. During the following summer, however, it was the writer's good fortune to be able to collect a series of stages of the large piddock, or "boring clam," *Zirphaea crispata* from the Pacific shores of the United States.[1] As the maturation and fertilization phenomena in the Lamellibranch also were at that time wholly unknown, the investigation of *Zirphaea* was immediately undertaken in connection with further study of *Thalassema*.

The work upon the achromatic elements produced results so clear and convincing that the writer was encouraged to take up a study of the chromatin with reference to the reduction question, which was then, as now, in fully as unsettled a condition as that respecting the centrosome. It was in this portion of the research that *Zirphaea* yielded the most important evidence, furnishing in fact the key to the understanding of the process in *Thalassema*.

dissertation for the degree of Doctor of Philosophy just before his last illness. Besides slight verbal alterations, which have not modified in any essential way either the substance or the form of his conclusions, the only changes that have been made are omissions of detail and the insertion of references to the figures. No literature-list accompanied the manuscript, and it has seemed best not to attempt the preparation of one. The text-figures have been prepared from sketches made as memoranda on the margin of the manuscript; but the author's methods of work were so conscientious that their accuracy may safely be assumed. An earlier and briefer paper on *Thalassema*, containing some details not included in the present work, was published by Mr. Griffin in the *Transactions N. Y. Acad. Sci.*, vol. xv, June, 1896, under the title "The History of the Achromatic Structures in the Maturation and Fertilization of *Thalassema*." I am indebted to Mr. Henry E. Crampton, Jr., for revision of the proofs of the present paper. — Edmund B. Wilson.

[1] *Cf.* Harrington and Griffin on Distribution and Habits of Some Puget Sound Invertebrates," *Transactions of N. Y. Acad. Sci.*, March, 1897.

In accordance with the above, the problem to be first attacked was that of the centrosome, — its morphology, its physiological significance, and its behavior towards fixing agents; on all of which points the accounts of previous investigators are, in a greater or less degree, conflicting. It was especially hoped that some positive evidence might be obtained respecting the continuity of the centrosome, a question which has of late seriously engaged the attention of cytologists. In opposition to the earlier view of van Beneden and Boveri, a considerable number of later authors (*e.g.*, Bürger, '92; Watasé, '94 and '97; Farmer, '96; Foot, '97; Carnoy, '97; and numerous botanical investigators) regarded the centrosome as a more or less transient structure, arising by the modification of preëxisting cytoplasmic elements (*e.g.*, microsomes, Watasé, '97), persisting for a shorter or longer period, and being dissolved and reformed according to the varying conditions of mitotic activity — the expression rather than the cause of the aster-formation (Foot, '97). On this point my results are, I think, clear and convincing as far as they go, though they certainly do not bring the question to a conclusion. In both *Thalassema* and *Zirphaea* the centrosome (sperm-centrosome in *Thalassema*) can be traced uninterruptedly from the first appearance of the aster throughout *all* intermediate stages into the cleavages, and at no time disappears from view. My results here agree entirely with those of van Beneden and Boveri (*Ascaris*), Mead (*Chaetopterus*), Wheeler (*Myzostoma*), Kostanecki and Wierzejski (*Physa*), and others. Neither in *Thalassema* nor in *Zirphaea* could the centrosomes be demonstrated during the growth period of the ovum, but it must be borne in mind that in the absence of rays or characteristic envelopes, a granule so minute as the centrosome would be indistinguishable from numerous cytoplasmic and yolk granules which fill the egg.

A third and no less important question touches the derivation of the cleavage-centrosomes, on which point my observations in the case of *Thalassema* have, I think, no room for doubt, while in the case of *Zirphaea* the facts are too equivocal to justify a positive conclusion.

In the fourth place, by reason of the varying accounts of different authors respecting the tetrads or quadruple groups, the nature of the so-called maturation divisions is still involved in doubt. Boveri ('87) (ovocyte of *Ascaris*) and Brauer ('93) (spermatocyte of *Ascaris*), ('92) (ovocyte of *Branchipus*) agree that the tetrad arises by a double longitudinal splitting of the spireme thread, thus giving no reduction in the Weissmannian sense. Other investigators, however (notably vom Rath, '92 and '94, in insects, and Rückert and Häcker in copepods), describe the process as consisting of one longitudinal and one transverse division of the primary chromatin rod, thus giving the theoretically required reduction. Here the *numerical* reduction (pseudo-reduction of Rückert) results from a suppression (or, more properly speaking, a postponement) of one-half of the transverse divisions of the spireme thread. According to some authors, however (*e.g.*, Wilcox, '95; Korschelt, '95; and Calkins, '95), the spireme segments into the normal number of chromosomes, and the numerical reduction is accomplished by a subsequent conjugation or association in pairs of the chromosomes. In some types, again (notably many plants, Elasmobranchs, etc.), no tetrads occur, and the chromosomes persist in their original rod-shaped or ring form. A number of more recent authors, working upon these forms, fail to find any evidence of reduction, and believe the maturation divisions to involve simply two successive longitudinal divisions of the chromosomes (*e.g.*, Farmer, '95, in plants; Meves, '96, in the spermatocytes of the salamander; and quite lately, Miss Sargant, '97, and Strasburger, '97, in *Lilium*).

The reduction question has been so often reviewed of late, that I may dispense with detailed references to the work of others at this point. My own observations, I believe, leave no reasonable doubt that in both forms studied a true reducing division occurs, and in this respect the Echiuroids (*Thalassema*) and Lamellibranchs (*Zirphaea*) fall into line with the insects and copepods.

I wish to take this opportunity to express to Prof. E. B. Wilson my sincere appreciation of his interest, advice, and continued encouragement.

METHODS. In both forms the material included stages fixed with picro-acetic (1%–2% acetic acid), sublimate acetic (20%, 10%, and 3% acetic acid), and pure sublimate. In general, the picro-acetic series gave the best results, while in *Zirphaea* the pure sublimate was a complete failure. The eggs were imbedded in paraffine cut into sections 2–10$\mu$ thick, mounted and stained on the slide by various stains. For the achromatic structures, by far the best results were obtained with iron haematoxylin, either alone or followed by acid fuchsin, Congo red, orange, or eosin. For study of the chromatin, the sections, after remaining in the haematoxylin for thirty-six to forty-eight hours, were extracted until the cytoplasm became colorless, and then restained with one of the plasma stains just mentioned. By this method the chromosomes stand out conspicuously as intensely black bodies upon a pale red or orange background. Their structure and changes can then be traced with but little trouble. Good results were also obtained by use of Flemming's triple stain and Auerbach's ('96) methyl-green acid-fuchsin mixture.

## PART I. — THALASSEMA

### I. THE OVARIAN EGG AND NUCLEOLUS.

The sexual products of Thalassema are developed, as Conn ('86) has described, from the peritoneal covering of one of the muscular bands about 5 mm. cephalad of the anus, and extending from the alimentary canal to the body wall. The ova and sperm-cells very early become detached in masses from the sexual glands, and undergo their subsequent development floating freely in the body cavity. My studies upon the early ova were made in large part from such masses imbedded and sectioned by chance along with the earlier maturation stages. These were not, however, in all cases to be relied upon, since degeneration had, in many instances, already set in. More satisfactory material was obtained by fixing the coelomic fluid, whereby many such egg masses were obtained along with innumerable coelomic corpuscles.

The earliest clusters obtained consist entirely of minute cells 3$\mu$ in diameter, all possessing extremely little cytoplasm and with nuclei in the spireme stage. After the segmentation of the spireme into the reduced number of chromosomes (*viz.*, 12), the ovum commences to grow and all parts rapidly increase in size, although the cytoplasmic growth is by far the

most rapid. In all these stages, up to eggs 47$\mu$ in diameter, the cytoplasm shows an extremely dense reticulum with no yolk-spheres. As growth continues, these appear in increasing number in the meshes. It would, therefore, seem that growth results as a distention of the reticulum by deposition of deutoplasm-spheres in the meshes, as in *Chaetopterus* (Mead, '97).

In mode of growth, the ova of *Thalassema* present some interesting resemblances to those of the allied genus *Bonellia*, though differing in several important particulars. In *Bonellia*, as Vejdovský ('78) has most clearly shown, the eggs arise from a peritoneal fold, and breaking away in clusters, undergo their subsequent development floating freely within the body cavity. But *one* (the central) cell of each cluster develops into an ovum, the remainder functioning as nutritive and follicle cells. Vejdovský shows that the egg develops at the expense of the nutritive cells, where the follicle cells become flattened and give rise to an outer egg membrane. In *Thalassema* only the peripheral cells develop into ova, though the precise fate of the more central ones is difficult to determine. Differential staining alone yields no evidence that they contribute in any way to the growth of the eggs. With Auerbach's acid-fuchsin methyl-green stain ('96), the reactions of the nuclei and cytoplasm are characteristic and opposite, the chromosomes and nucleoli taking a pure green, and the cytoplasm and nuclear reticulum a pure red. This yields equally little evidence that any of the chromatin is expelled from the nucleus to give rise to yolk material, as described by numerous observers (Blochmann, v. Bambeke, Balbiani, Calkins, and others). The cell outlines, especially those of the growing egg, are sharp, and the single hyaline membrane is distinct. There is here no follicular or cellular membrane, as in *Bonellia*.

*Nucleus.* — It is important to note that as the egg increases in size, the nucleus takes up an eccentric position on the side of the free surface of the egg (Pl. XXXI, Figs. 1–3). Thus the early, if not the ultimate polarity of the egg is to be referred to its position in the cluster, the attached side becoming the vegetative, and the free end the animal pole, as in *Cyclas* (Stauffacher, '93) and other forms. The bearing of this will

be seen later, when discussing the rotation of the first polar amphiaster.

The nucleolus was first noticed upon the breaking up of the spireme. Its intense blackness and rough and irregular outline give the impression of an irregular remnant of the spireme, and with Auerbach's fluid it can often be made to take a purer green than the chromosomes. The persistent nucleolus undergoes no change until the early prophase of the first polar mitosis. If it then happen to be near either of the asters, but not otherwise, it assumes an elongate ovoid outline, with the narrow end directed toward the center of the aster (Pl. XXXI, Fig. 11). Two portions are now distinguishable, a larger and more darkly staining area involving the pointed end, and a smaller cap-like portion at the blunt extremity. The two portions are not, however, to be compared to the Haupt and Nebentheil of the lamellibranch nucleoli, since they stain similarly and are not constant features. The nucleolus subsequently undergoes reabsorption along with the remnants of the nuclear skein (Pl. XXXI, Fig. 10).

## II. General History of the Achromatic Structures.

The egg of *Thalassema* is in many respects exceptionally favorable for cytological study. It is large (70–80$\mu$ diameter) and transparent, and the yolk is distributed in the form of large deutoplasm-spheres in the meshes of an extremely coarse cytoplasmic reticulum (Pl. XXXI, Figs. 10–12). The yolk distribution is, furthermore, such as to leave a relatively unobstructed field for the play of the mitotic phenomena, and in consequence the achromatic structures reach a powerful development, and comparatively little difficulty is experienced in following the history of the centrosome.

### a. *The First Polar Division.*

*Origin of the Amphiaster.* — The earliest stage that yields unequivocal traces of centrosomes is found among preparations of eggs fixed three minutes after fertilization. Their presence is then revealed by two excessively minute asters situated close

to the wall of the germinal vesicle, and about 45–90 degrees from each other (Pl. XXXI, Fig. 7). The rays of each aster converge directly to a minute dark-staining granule, the centrosome, which may at times be double even in this early stage. I find no certain indication of a rupture of the nuclear membrane behind the centrosomes, such as Mathews ('95) finds in *Asterias*, indicating their possible nuclear origin. The slight flattening or folding of the membrane seems to be rather the effect of inwardly growing rays, since it becomes steadily more marked as the asters develop. Not infrequently in these early stages one aster seems a trifle more advanced than the other, since the membrane behind it is considerably folded, while behind the other it is only flattened or but slightly folded (Pl. XXXI, Fig. 7). This inequality soon disappears.

In later stages the asters remain the same distance apart and undergo no further divergence. This is quite at variance with what occurs in most other forms. Platner ('89) (*Aulastomum*), Korschelt ('95) (*Ophryotrocha*), Wheeler ('95) (*Myzostomum*), Garnault ('88) (*Helix*), Mathews ('95) (*Asterias*), and others, all describe diverging daughter-asters or centrosomes immediately derived by division from a single one. It apparently agrees with *Thysanozoon* (Van der Stricht, '94) and *Chaetopterus* (Mead, '97). The definitive spindle, in consequence, arises in all cases secondarily by the meeting of rays from the opposite and independent asters, as in *Myzostoma* (Wheeler), Salamander (Drüner, '94), Selachians (Moore, '95), Opisthobranchs (MacFarland, '97), and some other forms.

*Centrosomes.* — In stages earlier than two to three minutes after fertilization, a most careful search has failed to reveal any structure that can, with certainty, be identified as a centrosome or definitive aster. The unfertilized, and some of the early fertilized eggs, however, exhibit an interesting phenomenon, which, from its possible bearing upon certain facts recently brought to light by experimental and other investigators (*e.g.*, Reinke, '94; Hertwig, '96; Mead, '97; Morgan, '96; Osterhout, '97; Mottier, '97) well merits description. In preparations of both full-grown and sometimes younger ova the strands of the cytoreticulum show a tendency to arrange themselves in radiating

lines, giving rise at various nodal points to aster-like appearances (Pl. XXXI, Fig. 6). Where the strands converge to a point between two microsomes, there is no focal granule, but when they focus upon a granule (microsome) the latter is often larger and more deeply stained than the surrounding microsomes, and a centrosome is thus simulated. This tendency to form centers of radiation seemed especially marked in a slide of eggs fixed by sublimate acetic (20%) while still within the sexual pouches. It may be interesting to note that where the eggs taken from the pouches have been allowed to remain in sea water for some time without being fertilized, this appearance becomes far less noticeable. Several preparations, one minute after fertilization, show numerous minute centers of radiation scattered irregularly throughout the cytoplasm, although more abundant or more powerful about the animal pole. At least ten were counted in a single section, some of which are closely approximated, while others are some distance apart. The rays are few (5 or 7), straight, and often short, though sometimes equal to or longer than the egg radius. These can sometimes be traced continuously without curving from one center to another. At the center a granule is generally present, which is distinguished from the neighboring microsomes by larger size and a deeper staining power. Similar granules, however, not the centers of radial systems, are to be met with throughout the cytoplasm. These "asters" are to be distinguished from the somewhat similar appearances in the unfertilized egg by their greater distinctness and straighter rays. In preparations made two minutes after fertilization the radiations are much more obscure, while in three-minute preparations and later, they are scarcely, if at all, traceable, their place being now taken by the definitive asters.

It is evident that we have here a phenomenon at bottom identical with that described by Mead ('97) in *Chaetopterus*. In *Thalassema*, however, the "secondary asters" are smaller and less distinct, and do not push in the nuclear membrane as they often do in *Chaetopterus* (*cf.* Mead). Moreover, I find no evidence of a fusion of the "asters" or of any genetic relation between them and the definitive polar asters, though it is not

improbable that *two* of the centers of radiation at the animal pole are caused by and belong to the true centrosomes.

In most cases the definitive asters appear on the side of the nucleus nearest the egg surface, *i.e.*, at the animal pole (Pl. XXXI, Figs. 7, 8); but sometimes they appear laterally (Pl. XXXI, Fig. 9), as in *Myzostoma*, and they may occupy any intermediate position. In such early stages I have never seen both at the vegetative pole, though occasionally one may occupy this position. From this it appears that the line joining the centers of the asters (the future spindle-axis) may be inclined to the egg-axis[1] at any angle from 0–90 degrees.

Considerable variation also exists in the distance separating the asters at their first appearance; for among eggs *fixed at the same period after fertilization and in which the asters have attained the same degree of development*, the angular distance was found to measure from 45–90 degrees, or more. This produces a variation, not only in the length of the completed spindle, but also in its position relative to the germinal vesicle. In extreme cases, when the angle is very small, the main rays may meet without penetrating the membrane, and thus results a short spindle wholly without the nucleus and quite near the surface of the egg. In this case it is the more lateral rays that enter the nucleus and come into relation with the chromosomes. The larger the angle, the longer and more centrally situated is the spindle. When, as in most cases, the angle is great enough to render the line joining the centrosomes secant to the nucleus, then the median rays, which are destined by meeting equatorially to form the spindle, push in the membrane and enter the germinal vesicle. In this case the completed spindle is more or less completely surrounded by the discarded chromatin-skein (Pl. XXXI, Fig. 10). This variation is of interest as bridging, to some extent, the gap between such cases as the salamander spermatocytes, where the amphiaster lies wholly without the nucleus, and forms like *Ophryotrocha* (ovum), *Ascaris* (spermatocytes), etc., where the nucleus comes to lie midway between the asters (*cf.* Drüner, '95, and Braus, '95), and would seem to indi-

[1] By "egg-axis" is here meant the line joining the centers of the egg and of the germinal vesicle.

cate that the difference between these extreme cases may be primarily only that of position of the asters.

Still another fact has to be considered as influencing the length of the spindle. By average of actual micrometer measurements (full account being taken of the above-mentioned variation in the distance between the asters) it was found that the completed spindle is approximately one-fourth *shorter* than the distance between the centrosomes previous to meeting of the rays — *i.e., the asters approach at completion of the spindle.* This often causes the asters to become more or less imbedded in the nuclear skein. A shortening of both maturation-spindles occurs in *Ascaria* (Boveri, '87), *Artemia* (Weismann und Ischikawa ('88), *Ophryotrocha* (Korschelt, *l. c.*), and *Branchipus* (Brauer, '82), and is explained by the first and last named authors as a result of contraction of the spindle-fibers. In *Thalassema*, however, the shortening takes place at a much earlier stage, probably while the chromosomes are still severally connected with but one of the asters. It more nearly resembles MacFarland's ('97) account of the approach of the cleavage-asters to form the cleavage amphiaster in *Pleurophyllidia.* Erlanger ('98) has recently described a shortening of the cleavage-spindle just previous to metaphase in the sea-urchins.

From its completion (Pl. XXXI, Fig. 12) until the polar body is separated, the first maturation-spindle maintains very nearly a constant length, measuring about $\frac{1}{3}$ the egg-diameter. A few measurements made during the expulsion of the polar body show that also during this process the spindle retains a fairly constant length, and hence must rise bodily with the elevation of the protoplasm that gives rise to the polar body. This has been also observed in *Physa* (Kostanecki and Wierzejski, '96). At this stage the egg, as a whole, elongates in the direction of the spindle-axis only $\frac{1}{2\frac{1}{3}}$ of the mean diameter.

The development of the asters, the progressive increase in the number, length, and strength of the rays which at first throw the nuclear membrane into folds and then rupture it and enter the nucleus (Pl. XXXI, Figs. 7–12), have all been described in a previous paper (Griffin, '96). During the early stages of the asters, while they are commencing to break through the

nuclear membrane, their rays are approximately similar in structure. As soon, however, as the chromosomes enter, in any considerable extent, into the system, certain rays commence to stand out more prominently. They are thicker, more deeply staining, than the remaining rays, and more homogeneous in that they are not microsomal in structure. As they can be traced to the chromosomes, they are evidently the developing traction-fibers ("Zugfasern") and are to be regarded as developing out of rays at first identical with the astral rays (*cf.* Drüner, '94). In both maturation-spindles these fibers stand out prominently, and in structure convey the impression as of threads spun of numerous strands. But a single traction-fiber is attached to each daughter-chromosome (Pl. XXXI, Fig. 12), as has been described by so many other observers in other forms.

Besides those attached to the chromosomes, the spindle contains numerous other fine and light staining fibers, which in some cases can be traced continuously from pole to pole. As there is here no distinction between *central spindle* and *mantle fibers*, these *central spindle-fibers* occur scattered throughout the entire spindle. In equatorial section they can be seen as numerous dots among which the chromosomes of the solid equatorial plate are interrupted.

*Movements of the Polar Amphiaster.* — In the stages previous to completion of the spindle, the line joining the centrosomes is very seldom situated in an egg radius. The completed spindle is, however, radial in position, with its aster still quite distant from the surface of the egg. It must, therefore, have undergone some rotation or change of position, however slight. Owing to absence of all means of orienting the spindle with reference to a fixed point of the egg, it was impossible to determine the precise extent of this shifting, though the occurrence of sections in which the spindle is oblique to the egg surface proves conclusively that it rotates to some extent. Sections through either aster of the just completed spindle show how completely the discarded nuclear skein has become involved in the astral system. The rays then appear to be but rearranged strands, the granules serving as microsomata (Pl.

XXXI, Fig. 11). Owing to this intimate connection, the discarded nuclear skein is, I believe, shifted along with the spindle, and there is, therefore, no means of orientation such as was found by Hertwig ('78) in *Asteracanthion*, where the spindle is figured as leaving behind the remains of the nucleus as it rises to the surface.

These considerations involve an important problem touching the polarity of the egg in relation to the cleavage-planes. We have seen (p. 588) that the eccentricity of the germinal vesicle, and hence that of the primary egg-axis, are to be referred to the position of the young egg in the cluster, the nucleus shifting toward the free side. If the rotation of the spindle is such as to bring it to always lie in the egg-axis, then the position of the polar bodies and the first cleavage-plane will, of course, depend upon the position of the young egg in the cluster. But another possibility is open. The outer aster remaining stationary, the spindle might rotate about it as a pivot just sufficient to bring the spindle-axis in an egg radius, in which case the position of the spindle, and hence that of the polar bodies and cleavage-plane, would be independent of the early egg-axis, and would vary according as the asters appeared in one or another of numerous possible positions about the germinal vesicle.

A rotation of the first maturation-spindle — generally through 90 degrees of arc — has been described in numerous forms. Thus Sobotta ('95) observed it in the mouse, Boveri ('87) in *Ascaris*, Weismann and Ischikawa ('88) in *Artemia* and *Eupagurus*, Brauer ('92) in *Branchipus*, etc. It appears, however, from the accounts of these authors, that it takes place in these forms much later than in *Thalassema*. Other observers find no evidence of rotation and believe the spindle to reach the surface by a motion of translation only, in the direction of its axis (*cf.* Korschelt, '95; Wheeler, '95; Kostanecki and Wierzejski, '96).

As the spindle rises, the outer aster soon encounters the membrane. The more outwardly directed rays in consequence steadily shorten, while the more lateral ones are deflected and curved backward — a phenomenon also well shown in *Physa* (Kostanecki and Wierzejski, '96). In the definitive position

(Pl. XXXI, Fig. 12) the outer aster is closely applied to the inner wall of the membrane and often flattened against it. The funnel-like depression of the egg surface next the outer centrosphere, not infrequently seen in other forms (notably *Physa* and the mouse), is here quite noticeable. It is probably to be referred to the influence of the aster, which causes the bounding surface of the egg to conform to the radial arrangement.

During the divergence of the daughter-chromosomes, the respective halves are united by fibers considerably finer than the traction-fibers, and which present a wavy and granular appearance (Pl. XXXI, Fig. 15). These interzonal fibers (Verbindungsfasern) are still present at telophase and can often be traced from the chromosomes in the extruding polar body to the corresponding ones within the egg. They soon disintegrate and break up into separate granules in the equatorial region, though a cell-plate (zwischenkörper) is formed.

### b. *The Second Polar Division.*

In mode of formation the second maturation amphiaster presents a marked contrast to the first. It arises shortly after the chromosomes have reached the poles of the first polar spindle, and apparently as a new formation between the already diverged daughter-centrosomes in the previously clear and homogeneous centrosphere (*cf.* Boveri, '90; Mead, '95; Korschelt, '95; MacFarland, '97; and others). The chromosomes remain for some time on the outer side of the spindle, stretching in a curved line from pole to pole (Pl. XXXI, Figs. 15–18). From now on, the spindle steadily elongates until its definitive length ($\frac{1}{4}$ to $\frac{1}{6}$ the egg-diameter) is attained, and at no time shortens. A rotation through 90 degrees of arc brings it radial with its outer aster at the point on the egg surface at which the first polar body was expelled. At division the extremely coarse, straight, and homogeneous interzonal fibers are gathered together as the polar body is constructed off, but the resulting cell plate disappears shortly after separation of the body (Pl. XXXII, Figs. 19–26).

The inner centrosome has meanwhile divided exactly as though preparing for a third mitosis (Pl. XXXII, Figs. 19–26).

Such a mitosis, however, never occurs; for shortly after the chromosomes remaining within the egg have passed into vesicles, the centrosomes, with all trace of rays, disappear completely. The reconstructed egg-nucleus, which results from the close aggregation, if not complete fusion, of the vesicles (Pl. XXXII, Fig. 28), now advances, unaccompanied by any sort of aster or rays, to meet the sperm.

### c. *The Sperm-Amphiaster.*

The sperm-aster first appears about the time of second polar telophase, at the edge of the clear area surrounding the sperm-head, and, roughly speaking, on the side nearer the center of the egg. It contains a single focal centrosome to which the rays converge directly (Pl. XXXII, Fig. 19). In many cases a process staining the same as the sperm-head extends from the latter through the clear area to the center of the aster (*cf.* Michaelis, '96, and Kostanecki, '96, both of whom have described a similar phenomenon). When no such process can be demonstrated, the aster appears independent of the sperm-head. The early division and divergence of the centrosomes give rise to a minute amphiaster already some distance in advance of the sperm-head (Pl. XXXII, Figs. 26, 27). This distance varies considerably (*cf.* MacFarland, '97), as do also the relative time and amount of the divergence, which may occur before or after the sperm-head has become vesicular. The daughter-centrosomes occupy the apices of an elliptical clear area outside of which the rays are grouped, those at the apices being focused at the respective centrosomes, while the intermediate rays still converge to the previous center, a phenomenon not uncommon in other forms. In some preparations a cloudy, rod-like structure, which may be called the centrodesmus (primary Centrodesmus of Heidenhain, '94), was seen extending between the daughter-centrosomes (Pl. XXXII, Fig. 27), but this bears no relation to the definitive spindle, since it soon disappears and leaves the asters independent. The further development is marked by steady increase in the number, length, and strength of the rays of these asters and the approach of the now hemi-

spherical sperm-nucleus so as to lie closely applied to the amphiaster, with an aster at each pole (Pl. XXXII, Fig. 32). The application of the fused or more often incompletely fused egg vesicles to the base of the sperm-nucleus and their subsequent fusion give rise to a segmentation-nucleus with the asters at opposite poles (Pl. XXXII, Figs. 33–37).

When, as often happens, the sperm enters from the side, the axis of the amphiaster is more or less nearly parallel to the egg-axis, as marked by the polar bodies. With the progress of the sperm-nucleus along its curved copulation path, the amphiaster is gradually rotated so as to bring its axis (the axis of the future cleavage-spindle) perpendicular to that of the egg. The relative position of the germ-nuclei at contact depends upon the extent to which the rotation has already progressed. A tardy rotation may cause the egg-vesicles to be received first upon one of the asters (Pl. XXXII, Fig. 32. *Cf.* Brauer, '92, for same phenomenon in *Branchipus*). Eventually, however, the rotation is effected so as to bring the egg-nucleus *between* the asters in the normal copulation position.

In some forms (*e.g.*, *Ascaris*, Mouse, *Unio*, *Prostheceraeus*, *Pleuroyphyllidea*, and others) the astral systems completely fail to appear, or disappear entirely about the time of copulation of the germ-nuclei; and in others (notably the sea-urchins) the rays become far less distinct, and a resting-stage results. On account of the extreme difficulty of detecting the centrosomes in these stages, such facts as the above have been considered by some observers as strong evidence against the persistence of the centrosome. In *Thalassema*, the "pause" is of short duration, and while the asters are a trifle less distinct, they nevertheless show clearly throughout, and the persistence of their focal centrosome is easily demonstrated. With the approach of the germ-nuclei the egg-vesicles seem to exert a disturbing influence upon the amphiaster, or render it more easily affected by reagents; for the astral centers are often somewhat distorted, the centrosomes pushed aside (carrying the asters with them) against the membrane of one of the nuclei or further out into the cytoplasm. In most instances the presence of the centrosomes can be made out with comparatively little diffi-

culty. With the commencing fusion of the nuclei, the centrosomes take up a polar position, and immediately become the centers of renewed activity, for many additional rays commence to start up about then.

From the above it is quite evident that *the centrosomes persist entire throughout the whole of the critical stage where, in so many forms, they have been lost sight of* (*cf.* Figs. 32–37).

### d. *The Cleavage-Amphiaster.*

After taking up a nearly central position, with its axis perpendicular to the egg-axis, the cleavage-nucleus rapidly elongates, sometimes even before the pronuclei have completely fused. Meanwhile a minute centrosphere becomes differentiated about each centrosome (Pl. XXXIII, Figs. 37–39), which has persisted unchanged up to this period, with the rays converging directly to it. The centrosomes remain single until shortly before metaphase, when each divides (Pl. XXXIII, Fig. 39); but the daughter halves do not diverge to any extent. The inner rays soon commence to push in the membrane at the poles, and later throw it into folds (Pl. XXXIII, Fig. 38) in the manner described by Platner (*Aulastomum*), Watasé (*Squid*), Braus (Salamander), and others. These in-growing spindle-fibers are essentially similar in behavior to those of the first maturation-asters. Like the latter, they are first similar to the remaining astral rays, but among them traction-fibers are soon distinguishable by their greater thickness and homogeneity (Pl. XXXIII, Figs. 39–41). For some time after the nucleus has become fusiform, with the chromatin-spireme crowded together equatorially, the membrane still persists laterally and conforms strictly to the astral system. Later its two halves merge into and become indistinguishable from its rays.

Equatorial sections during metaphase (Pl. XXXIII, Fig. 40) show the chromosomes arranged in a solid plate set in the center of a circular area of granules (cross-sections of spindle-fibers and those astral rays that pierce the equatorial plane), in all respects similar to the first maturation-spindle, save the double number (24) of chromosomes. Immediately upon divergence

the daughter-chromosomes are seen to be united by wavy and very granular interzonal fibers (Pl. XXXIII, Fig. 42), most of which later disintegrate (*cf.* Wilson, '95), though after the cleavage-furrow is complete, a few of these are still present and converge to several black granules in the furrow or to a single small black cell-plate (Pl. XXXIII, Fig. 46).

No sooner is the equatorial plate fairly established than the cloudy area, with its contained centrosomes, commences to migrate toward the outer periphery of the centrosphere where the cloudy area, having grown much less distinct, finally fades out entirely (Pl. XXXIII, Fig. 41). The centrosomes now diverge, and by mid-anaphase set up a minute amphiaster superimposed upon the crown of astral rays (Pl. XXXIII, Fig. 43). Aside from a slight elongation, it persists in this condition during the rest of the anaphase, the formation and fusion of the vesicles, and throughout the persistence of the reconstituted daughter-nucleus (Pl. XXXIII, Figs. 43–46). The centrosomes, by continued divergence, finally reach opposite poles of this nucleus, and the prophase of the second cleavage is thus initiated. Every step in this whole process can easily be followed out in detail, and there can be no question that the centrosomes persist throughout.

The initiation of a new and independent astral system under the apparent stimulus of the centrosome, while the persisting rays of the old still converge to a point previously occupied by the centrosome, is a fact noted by other investigators, although it has not, I believe, received the attention it deserves. Besides instances figured by Boveri ('90), MacFarland ('97), and others, the most striking case is perhaps that of the trout, as figured by Henneguy ('91). Here, within the old centrosphere, a minute aster is developed about each of the diverging daughter-centrosomes. Outside the long astral rays of the old system still converge toward the center of the former centrosphere. While he does not emphasize the point in the text, a comparison of his figures clearly brings out the migration of the daughter-centrosomes to the outer periphery, exactly as in *Thalassema*. Such facts as the above, I believe, lend the strongest possible support to the theory of the centrosome as an active stimulat-

ing agent in mitosis rather than the mere "expression of cell activity."

As this is an important point, it will be well to treat it a little more in detail. So long as the center of a new astral system coincides with that of a previous one, the mere *persistence* of the focal granules accords equally well with either of the theories representing the centrosome ; for all are agreed that such a structure, once formed, might well persist for some time. A crucial test would seem to be supplied by those cases in which a new system is formed about a point more or less removed from the old center. If the centrosome is but an *expression* of the forces that give rise to the aster, we should expect to see it deserted *in situ* at the old center and a new one formed at the focus of the new aster. We have shown that quite the contrary is the case, the new aster starting up about the old centrosome. In this, *Thalassema* furnishes the strongest evidence, since the purposeful migration of the centrosomes takes place *before* the formation of the new aster. In thus accepting the *physiological* theory of the centrosome as an active originating agent in mitosis, we must, however, clearly bear in mind that it by no means necessarily implies the *morphological* theory that it is a permanent cell organ, — though the balance of evidence seems at present to favor the latter view as well as the former. If subsequent research should render the morphological view untenable, this would not, I think, invalidate the physiological theory ; though if, on the other hand, the physiological theory be proved untenable, the main support would thereby be withdrawn from the morphological theory. In other words, if it ever be proved unequivocally that a centrosome can arise *de novo*, it would not thereby follow that the granule thus formed is solely an *expression* or by-product of the aster formation and not an active agent.

In all stages previous to the establishment of the equatorial plate, the astral rays can, in favorable preparations, be traced directly to the centrosome, the centrosphere, when present, being constituted by rays that stain more lightly and lack the grossly microsomal structure. From metaphase onward, however, a fine light-staining reticulum becomes developed in the

centrosphere between the rays and gradually replaces the latter. By the early anaphase, during the peripheral wandering of the centrosomes, the centrosphere has assumed a strictly reticular appearance, as described by Wilson and others in the Echinoderms (Pl. XXXIII, Figs. 41, 42).

This progressive development of the reticulum *pari passu*, with decrease of rays within the centrosphere, and following close upon metaphase, constitutes strong evidence that the reticulum is developed out of the substance of the rays; and this evidence is all the stronger from the fact that both rays and reticulum stain exactly the same. We have here a radial structure breaking up into a reticular one. But in cells of this type the radial structure is indicative of mitotic activity, the reticular of repose ("resting" condition). These considerations lead us to the view that the reticular centrosphere is a degeneration phenomena consequent upon the withdrawal (or cessation of activity) of the centrosome to initiate a new system, and heralds the breakdown of the astral system. From now on, the old system with its rays actually does disintegrate. Evidence drawn from comparison of other forms lends additional support to this view, for in all cases, as far as I know, the reticular centrosphere is secondary and preceded by stages in which the rays focus directly to the centrosome or its more *immediate* envelopes.[1]

The enormous number of rays in the dense astral crown during anaphase renders it impossible to regard them as entirely consisting of rearranged cyto-strands, as Wilson and others have so interpreted them. As there is obviously insufficient material in the preëxisting cyto-reticulum for this purpose, we are forced to look upon them as in part a new formation. The additional material is probably derived as a product of some specific form of metabolic activity set up by the centrosome, while their radial arrangement is most likely due to a radial disposition of the forces thereby disengaged, more or less analogous, though of course not strictly comparable, to the magnetic "lines of force."

[1] *Cf.* Sea-urchins (Wilson, Kostanecki, and others); *Nereis* (Wilson); *Chaetopterus* (Mead); *Triton* (v. d. Stricht, '92); Trout (Henneguy, '91); *Rhynchelmis?* (Vejdovský), etc.

## III. The Chromatin.

### a. *In Maturation.*

Among the early egg-clusters are some in which the chromatin of every cell is arranged in a thick, rough, dark-staining spireme, in which occasional indications were observed of the longitudinal splitting so frequently observed in other forms at this period. It is impossible to say with certainty whether this spireme consists of a single thread or several — a difficulty that has frequently confronted other observers. It is coiled in several turns about the nuclear membrane, against the inner wall of which it seems to be principally situated (Pl. XXXI, Figs. 1, 2). Lack of earlier stages has prevented the working out of the genesis of this spireme. In later egg-clusters are cells containing a spireme in all respects similar to the above-described, except the commencing transverse division and marked indications of the longitudinal fission (Pl. XXXI, Figs. 1, 2). In other cells of the same cluster this process has advanced one step further, the spireme being now completely divided into segments connected by numerous linin strands (Pl. XXXI, Figs. 3–5) and partially cleft longitudinally to form stout and much-flattened rings. The transverse division appears to be followed by a shortening and concentration of the resulting segments, obliterating their granular appearance (see Häcker, '95, *Canthocamptus*, for similar phenomenon). Although the exact number of these segments is not easy to determine, they are readily seen to be nearer twelve than twenty-four, which clearly indicates that the chromosomes first appear in the reduced number as in so many other forms.

Meanwhile a fine nuclear reticulum is gradually developed, which readily takes the plasma stains, and could not be made to take the pure chromatin stains. Throughout the entire growth period it increases in bulk and distinctness until, in the full-grown ovum, it fills the entire germinal vesicle (Pl. XXXI, Figs. 3–6). During prophase of the first maturation division, after exhibiting a marked increase in its affinity for iron haematoxylin, it is rejected bodily by the spindle and degen-

erates in the cytoplasm (Pl. XXXI, Figs. 10, 11). The source and mode of growth of this reticulum are impossible to determine. It evidently does not arise through an anastomosing of chromosome processes as in the germinal vesicle of *Pristiurus* (Rückert, '92) and epithelial cells of Salamander (Rabl, '85); for all the facts suggest that its development is independent of the chromosomes which are passive during its growth. I would conclude, therefore, that this nuclear reticulum is a secondary and special structure, developed to preside over the metabolic functions of the egg during the growth period, when the true chromatin (idioplasm), in the form of chromosomes, is passively awaiting the formation of the spindle.[1] This view may be elucidated by a comparison with the Infusorian nuclei. It is here generally conceded that the macronucleus is the more especially concerned with the purely vegetative functions of the animal, while the micronucleus represents a reserve idioplasm especially concerned with reproduction. Exactly such a difference in function I conceive to exist between the nuclear reticulum and the chromosomes in ova of the *Thalassema* type. The following rough parallel may be drawn:

| INFUSORIAN. | EGG-CELL. |
|---|---|
| 1. *Vegetative period*, when the cell is dominated by the *Macronucleus*. | *Growth period*, presided over by the *nuclear reticulum*. |
| 2. Macronucleus breaks up, disappears, to be followed by | Nuclear reticulum breaks up, disappears, to be followed by |
| 3. Division and persistence of Micronucleus. | Division and persistence of the Chromosomes (polar mitosis). |

Up to this stage my results on the tetrad-formation agree with those of vom Rath in *Gryllotalpa*. Instead, however, of immediately concentrating into tetrads, as in *Gryllotalpa*, the rings now begin to elongate, and their granular composition becomes very apparent. In full-grown ova within the sexual pouches they appear as small, much-coiled, or zigzag, granular rods (Pl. XXXI, Fig. 6), generally near the membrane, though sometimes a few are more nearly central. This coiled or zig-

[1] In the Selachians it would appear (Rückert, '92) that its function is assumed by the true chromatin, a similar reticulum arising by the chromosomes putting forth anastomosing processes, and losing their affinity for chromatin stains.

zag condition greatly obscures their true form, but in favorable cases they appear double. In other preparations of unfertilized eggs removed from the pouches, the chromosomes stand out very plainly as black and often clearly double rods.[1] In thus demonstrating the presence of the chromosomes as double rods throughout the entire growth period, my results agree with those of Häcker ('92), Rückert ('92), vom Rath ('92), and others.

The chromosomes persist in the above-mentioned shape until the asters commence to break through the wall of the germinal vesicle, when they seem to undergo a concentration and approach the asters. In entering the spindle, the chromosomes sometimes arrange themselves about the distal ends of the inner rays, simulating the daughter-chromatin plates of an anaphase (Pl. XXXI, Fig. 10). By growth of the rays these two groups become pushed together, meeting equatorially to form the equatorial plate. Bolles Lee ('97) has described a similar phenomenon in *Helix*.

The nuclear reticulum now shows a marked increase in its affinity for haematoxylin, which increase continues until after completion of the spindle; the stain is retained with a tenacity not surpassed by even the chromosomes or the centrosomes. After complete extraction of the haematoxylin from the cytoplasm, and replacement by Congo red, the discarded reticulum appears as a dense, thick-stranded, dark blue or black skein upon a red background (Pl. XXXI, Fig. 11). After Flemming's Triple, however, it is indistinguishable from the surrounding cytoplasm, though after Auerbach's fluid, it takes the *fuchsin* rather more deeply than the cytoplasm. The skein gradually undergoes resorption, decreases in bulk and distinctness, and finally fades out entirely, though traces of it are sometimes met with as late as first polar metaphase with peripherally situated spindle.

While entering the spindle in the prophases, the chromosomes exhibit a great variety of forms, which in most cases are

[1] It was these irregular black chromosomes that I described (Griffin, '96) as "here and there . . . light thickenings or bunching together of strands of the chromatic reticulum, which is doubtless a prelude to chromosome formation." More extended study of later and earlier stages now shows them to be much-twisted ring or rod chromosomes.

easily reduced to the type of a double rod. In its simplest form this rod consists of slender halves terminating at each end in a common knob or swelling (Fig. I, *a*). In drawing apart in the middle, the halves may give rise to a knobbed ring (Fig. I, *b*), and by a continuation of this process a perfect ring arises, with or without one or two bead-like swellings (Fig. I, *c*, *d*).

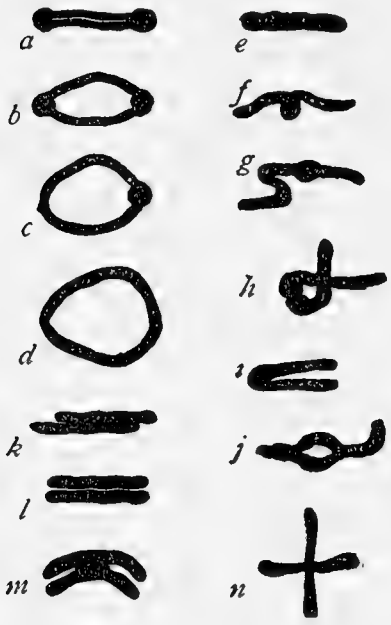



Fig. I.—Formation of chromosome groups (tetrads) in the prophases of the first maturation-division in *Thalassema*; *a–d*, ring formation; *e–j*, apparently single-rod type; *k–n*, double-rod type.

These rings may be open and simple, or variously coiled or twisted into the shape of a figure 8. Occasional slender rods, of extreme thinness and variously coiled or bent, are most readily interpreted as segments of rings cut or broken by the knife. In most cases the granular nature of both these latter rings and rods is quite apparent. Similar figures have been described by Kastschenko ('90) and Rückert ('92) in the Selachians, Fick ('93) in the *Axolotl*, Häcker in Copepods, Farmer and Moore ('96) in *Lilium*, and others.

Shorter, much thicker, and often apparently homogeneous rods also occur, which may be very short (Fig. I, *e*) or longer and generally with a central swelling (Fig. I, *f*). These may be fairly straight or variously coiled, or even bent into more or less of a *V* shape (Fig. I, *g*, *h*, *i*). In certain favorable cases, clear evidence of a *double character* appear, as a distal forking or breaking apart of the halves in the center (Fig. I, *j*). From this fact, as well as from study of later and earlier stages, and analogy with *Zirphaea*, I do not hesitate to consider all these varied forms double rods in which the halves are so closely appressed that the line of separation is obliterated. The central knob

is probably the beginning of a process that will be treated in detail after the description of the morphology of the chromosomes in metaphase. From the complete failure to find forms indicating a direct transformation of the open rings into the metaphase figures, it seems not improbable that the thick rods may represent a later and more concentrated and compressed stage of the rings. This also appears to be indicated by measurements which show the rings to be of greater length than the rods. The open ring, however, is not to be considered a necessary stage, for it is quite evident that many of the rods arise directly from the similar structures present in the germinal vesicle.

More difficult to determine are the occasional two short rods lying side by side (*k*, *l*, *m*), as well as crosses or ophiurid forms (*n*), the arms of which appear perfectly solid without the slightest indication of a division into halves. A few may possibly be explained by supposing an accidental adherence of two distinct chromosomal rods. Others may have resulted from a premature division, either longitudinal or transverse, of a single rod, and the subsequent rotation of the halves; or the cross might be considered a ring compressed along two perpendicular diameters, whereby the four included quadrants were converted into loops. The close adhering of the halves of these loops might well obscure the line of separation and cause the arms to appear solid. Analogy with *Zirphaea* would strongly suggest the last-mentioned view.

Despite the varied forms presented during prophase, the chromosomes of the equatorial plate exhibit considerable uniformity. Hence the various prophase forms must in some manner be convertible into a uniform type of metaphase figure. The commonest and, as we shall consider it, the typical form assumed by the chromosomes in the latter stage is that of a cross, with a pair of broad arms in the equatorial plane, and the narrower perpendicular arms directed toward the poles of the division figure (Pl. XXXI, Fig. 12, Text-fig. II, *o*, *p*). The latter pair or polar arms are of varying length, and invariably end distally in a solid knob, to which a single traction-fiber is attached. The more proximal portion is often seen

with greater or less clearness to be divided into halves by a pale longitudinal streak extending along the middle. Viewed laterally, these arms are quite thin, and invariably single (Pl. XXXI, Fig. II, *r*). The broad lateral arms, also of varying length, appear in general quite solid, although occasionally what seemed like a similar streak was observed to divide them in halves (*p*). Rarely straight, and in the same plane as the polar arms, they are more often curved or bent toward each

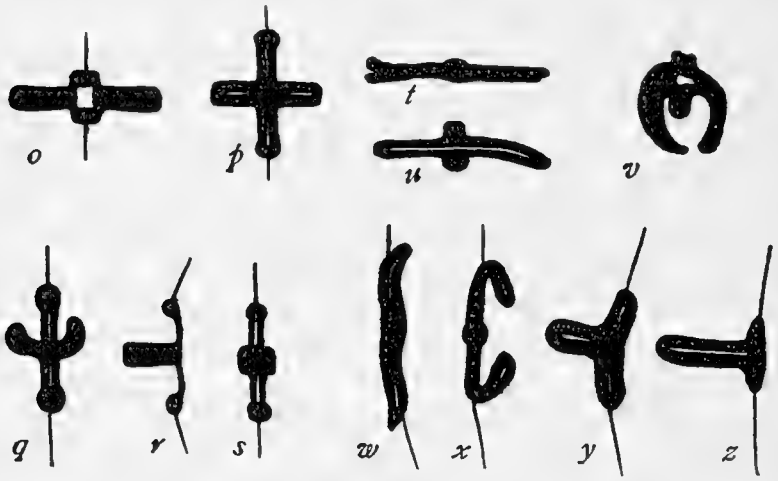



Fig. II.—The chromosome groups (tetrads) of the first maturation-division in *Thalassema*. The narrow lines represent the traction-fibers; *o*, early, *p*, late typical cross; *q*, curved cross; *r*, curved cross with fused lateral arms in lateral view; *s*, the same seen from above; *t*, *u*, origin of the cross from rod-form; *v*, early stage of curved cross; *w–z*, unusual and asymmetrical forms.

other (*q*, *v*). By increased bending, the arms may come into contact, whereby a chromosome, *T*-shaped, in lateral view results (*r*, *s*). Similar *T*-shaped chromosomes, but without the distal knobs, are figured by Fick ('93) in the corresponding division in the *Axolotl*. Moore ('95) also obtains not dissimilar figures in the *Elasmobranchs*, and interprets them in an analogous manner.[1]

[1] The possibility is not *completely* excluded that these *T*-figures may be due to a second longitudinal division of the ring, the two double halves immediately diverging. In view, however, of the prevalency of the crosses and other shapes, in which a second longitudinal division is excluded, it seems more probable that the above interpretation is the correct one, unless, indeed, we accept the possi-

The crosses are to be derived from the double rods by a swelling or looping up of the halves in the middle. The beginning of the process is represented by the previously described thick rod with central bead-like swelling (Fig. II, *f*). In a slightly later stage, a central hollow appears which, separating the swelling into two knobs, shows the rod to be double (*o*, *u*). In the form figured at the rod is greatly curved, but it may sometimes be straight, when a dagger-shaped figure results (*o*, *u*), often with a clear central split. In this respect also it differs from Klinckowström's figure of a dagger-like chromosome in *Prostheceraeus*. The metaphase crosses, with curved lateral arms, may have resulted from rods curved like *i* or *v*. A further increase in size of the lateral swellings produces a cross (*q*) quite similar to those of the equatorial plate.[1]

The chromosomes now being taken into the spindle (Pl. XXXI, Figs. 12, 13), it becomes of importance to determine whether the arms formed by the mid-swelling of the halves of the rod become the polar or the lateral arms. In the former case the axis of the double rod (which is that of the original spireme segment), being coincident with the equatorial plane, the first division would be an equation division. If, on the other hand, they become the lateral arms, the reverse would hold, and the resulting division would be a reducing division. The former I believe to be the correct alternative. For, in the prophases, the lateral swelling seems considerably shorter than the two other arms of the cross, while in early metaphase, the *equatorial arms* are often quite long, and the polar ones

bility suggested by Wilcox ('**95**), that in one and the same spindle some chromosomes undergo a reducing, and others an equation division. The occurrence of varied prophase chromosome figures, all of which subsequently develop into a uniform type of metaphase figure, recalls Calkins's ('**97**) results in the fern. In *Thalassema*, however, the end result is not a four-sphered tetrad (as in the fern), but a ring variously looped and, as we have seen, a careful comparison of all intermediate stages shows that the interpretation given by Calkins of the crosses in the fern does not hold in *Thalassema*.

[1] Considerable variation exists in the degree of approximation of the halves of these figures, and hence in the clearness with which the central split appears. Rarely it appears with great clearness, but more often it can be satisfactorily detected only by the very best optical means (*e.g.*, Zeiss, Apoch. 1.5 mm. oculars 6–12, with most powerful light), while sometimes even these fail to bring it out, and the arms then appear perfectly solid.

very short. From this it would seem that the longer axis of the prophase cross corresponds to that of the equatorial arms of the metaphase figure and hence the following division is an equation or longitudinal division.

As previously mentioned, the arms of the metaphase crosses vary extraordinarily in length, and may be of any size from a short knob to a long process. The variation, however, conforms to an obvious law, since the longer the lateral arms, the shorter the polar, and *vice versa*, as in *Lilium* (Farmer and Moore, '95). Moreover, those spindles containing the greatest number of crosses, with short lateral arms and short-bodied *T*'s, are the more advanced. The meaning of all this is very obvious; *the lateral loops are gradually unfolding, and their substance passing into the polar arms.* Throughout this process the central furrow, separating the halves of the polar arms, is sometimes clearly apparent, and sometimes not. The process continuing, the lateral arms steadily decrease in size, and with their disappearance the chromosomes round out to more or less of a ring, in which the central furrow is sometimes most clearly shown (Pl. XXXI, Figs. 13, 14). The chromosomes now divide in the equatorial plane.

Upon separation, the halves or daughter-*V*'*s* may become immediately contracted, and their bases squared off, or for some time may remain elongate and more or less drawn out. The split separating their limbs is sometimes very clear (Pl. XXXI, Fig. 15). During divergence they progressively shorten and contract into sausage-shaped rods, and at or even before telophase they break apart at the angle, and the limbs becoming entirely free, give rise to double chromosome of two short sausage-shaped rods lying side by side (Pl. XXXI, Fig. 16). However varied the prophase, or even the metaphase figures may be, the daughter-halves behave precisely alike in all cases examined, invariably dividing transversely at the apex of the *V*.

We are now in a position to understand certain, at first sight, aberrant chromosomes occasionally met with in the equatorial plate. Observed but once or twice in metaphase, is the doubly curved or elongate *S*-shaped rod (II, *w*), with a slight central

swelling, and the traction-fibers attached some distance from the distal extremities. Another form (II, *x*) is similar to one figured by Flemming in the salamander, and considered as a ring split at but one point. Here, however, it evidently has a different interpretation, being probably similar to the above, but under greater tension, and with the free distal ends bent further. Both show the faintest possible indication of a central split. These may be rings just previous to division and after the disappearance of the lateral loops, or those in which lateral loops have failed to develop. In the latter case they would divide transversely, while the remaining chromosomes split *longitudinally*, and an equation and a reducing division would occur simultaneously in the same spindle — a possibility urged by Wilcox ('95). A third deviation, observed but once, is the thick *T*-shaped form (II, *y*, *z*), in which the rounded extremities and faint streak in the center of each arm show it to be a ring with but one lateral loop instead of two. Occasionally the ring, failing to develop lateral loops, becomes attached to the traction-fibers, near one extremity (II, *z*), as Farmer and Moore ('96) figure in the pollen mother-cells of *Lilium*, except that it does not break apart at the attached end. The just-mentioned thick *T*-form may represent a more contracted condition of this ring, or may have been derived from one of the open rings.

After completion of the first division the dyads are taken immediately into the second polar spindle and arranged at right angles to the spindle-axis, with the halves directed toward either pole (Pl. XXXI, Fig. 18; Pl. XXXII, Figs. 19, 20). A single traction-fiber is attached to one end of each of the rods. In those on the periphery of the spindle, this is the *inner* extremity.[1]

The division of the daughter-*V's* in anaphase or telophase recalls *Ophryotrocha*, except that in *Thalassema* the halves remain associated in pairs, and it is certain that one member of each pair, and not a whole pair, passes into the polar body.

[1] In cases where the halves are closely appressed in the middle, and swollen at the extremities, an appearance suggesting tetrads sometimes results. This, I believe, is all the significance there is in "tetrads" described by some authors (*e.g.*, Klinckowström, '97) in the second polar spindle.

At anaphase the ends attached to the traction-fibers are the first to diverge, while the free extremities cleave together, producing a more or less open *V* (Pl. XXXII, Fig. 25). This opens further, the two limbs become straight and then separate, producing the appearance as though a single rod had just divided transversely, precisely as Wilson has described in the cleavage-spindle of *Toxopneustes*. The diverging halves maintain a pretty constant length, and are as long as the daughter-*V*'s in the first polar telophase. At telophase the chromosomes remaining within the egg pass into minute vesicles which, by fusion or close aggregation, constitute the egg-nucleus.

SUMMARY.

*1. By longitudinal fission and transverse segmentation of the spireme-thread, there arise 12 (reduced number) ellipse-shaped chromatin masses.*

*2. These persist throughout the growth period of the egg.*

*3. During prophase they concentrate into crosses, the arms of which are tight loops.*

*4. In the first polar division, these are drawn out again into ellipses which divide to form daughter-V's (equation division).*

*5. The V's break apart at the angle in the second polar division (reducing division).*

### b. *In Fertilization and Cleavage.*

In general, the sperm enters nearer the vegetative pole, though it occasionally penetrates above the equator.[1] After entrance it increases enormously in size, but remains not far from the surface as a large black sphere within a clear area (Pl. XXXI, Fig. 12; Pl. XXXII, Fig. 19). By second polar telophase, roughly speaking, it becomes vesicular (Pl. XXXII, Fig. 26) and ultimately gives rise to a fine reticulum like that of the egg-

[1] In one or two cases a sperm (probably a supernumerary one) had entered at the animal pole, near the outer aster of the first polar figure. A similar fact has been reported by Foot ('95) in *Allolobophora*, and Kostanecki and Wierzejski in *Physa*.

nucleus (Pl. XXXII, Figs. 32, 33). The more or less complete fusion of the germ-nuclei extends to the chromatin network of each, which even before the fading of the separating membrane shows dark-staining thickenings (Pl. XXXIII, Figs. 35, 36). The ingrowing astral fibers now crowd towards the equator, the chromatin (Pl. XXXIII, Figs. 37, 38), which, by this time, forms a more or less perfect spireme. Intervening strands of a finer and more granular nature (linin?) are, however, still traceable. With the complete disappearance of the nuclear membrane, the spireme becomes closely compacted in the equatorial plane (Pl. XXXIII, Fig. 39). Bead-like swellings occur at regular intervals along the entire length of the thread; these mark out the chromosomes. By transverse division at these swellings, there result 24 pin-shaped chromosomes, attached by their heads to the traction-fibers (one fiber to each chromosome). The whole spireme thus going over into the chromosomes, there is here no bodily casting out of chromatin. The above-mentioned finer strands probably represent portions of the original chromatic reticulum that break down into linin, as claimed by Wilson ('95).

The subsequent history, the divergence and conversion into vesicles of the pin-shaped daughter-chromosomes, the reconstitution of the nuclei and genesis of the second cleavage amphiaster (Pl. XXXIII, Figs. 41–46), does not appear to contain anything worthy of especial remark. At no time was any chromatin rejection observed such as Boveri ('92) has described in *Ascaris*.

## IV. The Polar Bodies.

The formation of the polar bodies in *Thalassema* and the almost invariable division of the first have already been minutely described by Conn ('86). As his researches were limited almost entirely to the living egg, he was unable to follow out the details of the mitotic phenomena. The latter are easily studied in sections. The first polar body divides by a complete and typical mitosis, although in minor details it shows evident signs of degeneration.

The constriction of the cytoplasm which separates the polar bodies does not commence until telophase, when the chromosomes are at the poles of the division figure (Pl. XXXI, Fig. 16; Pl. XXXII, Fig. 19). At this stage there commences to appear a small projection or knob of clear granular cytoplasm, entirely devoid of yolk-spheres and containing the outer group of chromosomes and the outer centrosome (or centrosomes). The knob increases in height and with a deepening of the constriction becomes balloon-shaped, connected with the egg by a narrow cytoplasmic bridge. Often this constriction advances more rapidly on one side, giving a slight obliquity to the polar body, as in *Limax* (Mark, '81) and in the corresponding spermatocyte division in Amphibians (Flemming, '87). With the severing of the bridge the body rounds out, leaving the surface of the egg rough and uneven, as described in *Ophryotrocha* (Korschelt, '95). The second polar body arises in an essentially similar manner.

Within the rising polar body the centrosomes and chromosomes take up an extreme distal position (Pl. XXXI, Figs. 15–18; Pl. XXXII, Figs. 19, 20). In appearance and behavior the centrosomes are in no way to be distinguished from those remaining within the egg. Early divergence, which may take place while the polar body is as yet represented by only a slight hummock (Pl. XXXI, Fig. 15), followed by the appearance of rays, gives rise to a minute amphiaster. Further divergence of the centrosomes is accompanied by an elongation of the polar body, which, in most cases, takes place tangentially, although sometimes, doubtless owing to a mechanical rotation of the polar body, the spindle lies with its axis in an egg-radius (Pl. XXXII, Figs. 21–25).

The history of the chromosomes is, in all essential points, but a repetition of that of those remaining within the egg. Soon after the polar body becomes separated, or even while it is connected with the egg (Pl. XXXI, Fig. 16), the line separating the limbs of the daughter-V's becomes very clear. A little later the limbs break apart at the angle and shorten up into sausage-shaped bodies associated in pairs (Pl. XXXII, Fig. 22). An equatorial grouping of these dyads follows upon sufficient divergence of the centrosomes, but as the individual dyads tend to

scatter some distance on each side of the equatorial plane, the result is by no means a compact and sharply defined "plate," as in the second polar spindle. The telophase (Pl. XXXII, Fig. 30) shows the chromosomes aggregated in a mass at each pole, and the commencing cytoplasmic division. The latter, however, may be retarded (Pl. XXXII, Fig. 31), or even fail to occur, in which case the two chromatin bunches remain at the poles (Pl. XXXII, Fig. 29). The centrosomes were only occasionally to be made out at telophase. Not infrequently in these stages a number of dark-staining bodies were observed in an equatorial position (Pl. XXXII, Figs. 30, 31) in all respects similar to those figured by Korschelt in *Ophryotrocha* ('95), and considered by him to be chromatin particles left behind in the anaphase.

After this division the three polar bodies remain clustered together, and may attempt the formation of resting-nuclei. In the two-celled stage one or more of them is to be found between the blastomeres (Pl. XXXIII, Fig. 46), evidently there undergoing degeneration and absorption. One or two centrosomes pass into the second polar body; but I have not observed the latter to divide or commence any of the initial stages.

A mitotic division of the first polar body is a very common occurrence, and has been repeatedly observed by previous investigators, though few attempts have been made to follow out the process in full detail. This is no doubt due to the minuteness of the elements and the often abortive nature of the mitosis. The earliest reference to a *division* of the first polar body appears to be attributed to Kölliker in 1852 (Fick, '93). Hertwig ('77) describes the phenomenon in the leech *Nephelis*, though unable to make out the behavior of the nucleus. From the mention of vacuoles that fuse into one, we are led to infer that a resting-nucleus is formed or attempted. No spindle nor centrosomes are figured. Trinchese ('80) observed the *mitosis* of the first and also of the second, as did also Blochmann ('89) in the honey bee, and Nussbaum ('89) in *Pollicipes*. Blochmann ('82) gives a very complete account in *Neretina*. He figures a spindle in metakinesis lying paratangentially with its axis in the direction of the greatest diameter of the body. No distinct centrosome is demonstrable. The chromosomes are described as uniting into "ein solider Kern." The anaphase with Verbindungsfäden is figured and also the cytoplasmic division. Platner ('86) finds the division of the first a constant occurrence in *Arion*, giving three bodies into which spermatozoa sometimes enter. He observed a spindle in

metakinesis and various stages in the division. Garnault ('88) studied both *Arion* and *Helix* and describes the first polar body as dividing mitotically with or without the formation of a resting-nucleus. In numerous insects (Blochmann, '87, '89, and Hertwig, '90) each of the two groups of daughter-chromosomes of the first maturation-spindle divide at telophase while still within the egg, giving rise to four groups of chromosomes. These pass into four resting-nuclei (the female pronucleus and three polar nuclei). Vejdovský ('88) has described the mitosis of the first polar body in *Lumbricus rubellus* and *Allolobophora foetida*. A completed spindle is figured of the latter, with one well-developed aster. The spindle lies in the greatest diameter of the polar body, which is here radial and not paratangential.

A mitotic division, or at least some of the initial stages, has been also noted in numerous insects (Blochmann, '87), in Amphibians (Schultze, '87), *Myzostomum* (Wheeler, '95), the Mouse (Sobotta, '95), *Phallusia* (Hill, '95), and other forms. Quite recently Korschelt ('95) has described, in some detail, the nuclear changes in the polar bodies of *Ophryotrocha*, in which considerable variation appears to exist.

From the above it appears that the phenomenon in question is a pretty general one; that in some cases it results in a complete division typically mitotic; but that more often the attempt is abortive and the process ceases with the attainment of some intermediate stage. In a few cases a resting-nucleus may intervene previous to the division.

## PART II. — ZIRPHAEA.

### V. DEVELOPMENT OF OVA.

The earliest ova that can be easily recognized as such have more or less oval nuclei, measuring about 6 by 9$\mu$ in diameter. They lie imbedded in the stroma of the ovarian tubules, with a minute quantity of cytoplasm heaped on either side. A cell membrane could not be demonstrated with certainty. The large, dark-staining nucleolus is single and eccentric. The nuclear reticulum is as yet represented by a few strands only. Peripherally situated chromosomes are occasionally to be observed. The ovum has clearly passed out of the spireme stage and has entered the growth period. During the growth period numerous nuclei that stain a brilliant green with Auerbach's fluid are present in considerable number, some scattered irregularly throughout the stroma, others closely appressed against the growing egg. They are evidently nutritive nuclei.

In the earliest stages the cytoplasm of the growing egg takes,

with Auerbach's fluid, a uniform bluish green, sometimes almost as brilliant as that of the nuclei. As the egg increases in size and protrudes into the lumen of the tubule, the cytoplasm takes the fuchsin in increasing quantity and assumes more and more of a reddish tint. This ultimately predominates over the green, and by the time the egg has broken away from the wall of the tubule the green is scarcely noticeable. Iron haematoxylin, followed by orange or other plasma stain, gives a similar result ; the cytoplasm, which in early stages takes the haematoxylin intensely, shows with growth a steady increase in its affinity for orange. These changes can hardly be due to expulsion of nuclear elements into the cytoplasm, for all elements within the nucleus show a steady growth and differentiation. The chromosomes and nucleolus increase in size, and the latter in complexity, while *pari passu* the formerly sparse and scattered nuclear reticulum becomes close and compact, filling the entire nucleus. Moreover, in some preparations, the base of the growing egg showed a more marked affinity for the nuclear stains than did the cytoplasm near the nucleus. These points, taken in connection with the behavior of the free nuclei, point to the latter as being the active agent in elaborating cytoplasmic material.

When full grown, the ovum is but half the diameter (*viz.*, 40$\mu$) of that of *Thalassema*. Its close and dense cytoplasmic reticulum, in whose meshes the fine granular yolk is distributed, renders the egg excessively opaque. The asters and spindles do not, in consequence, show as brilliantly as in *Thalassema*, while the centrosome is often lost to view among the innumerable granules that fill the egg. The germinal vesicle is quite eccentric and filled by a close reticulum. The complex double nucleolus (Pl. XXXIV, Fig. 47), characteristic of Lamellibranchs and some vertebrates (Flemming, '82), some annelids (Wilson, '96) and invertebrate liver cells (Lömberg, '92), here shows to advantage. The smaller portion ("Hauptheil" of Flemming) stains black with haematoxylin and green with Auerbach's fluid. Generally hemispherical in outline, it sits like a cap upon the larger vesicular "Nebentheil" (Flemming). It is, however, sometimes apparently spherical, or even bi- or

tri-lobed, as described by Stauffacher ('93), in *Cyclas*. The Nebentheil varies in appearance, according to the fixing-fluid employed. After picro-acetic it is large and clear, about three or four times the diameter of the Hauptheil, and contains a bunch of granules staining black with haematoxylin and red with Auerbach's fluid. After sublimate acetic, and occasionally also after picro-acetic, it is larger, bordered by a denser and more deeply staining zone of the nuclear reticulum, and is filled with a finer and sparser reticulum, which shows even greater affinity for plasma stains than does the nuclear reticulum. From the accounts of numerous authors, it would appear that this latter appearance represents more nearly the normal condition, and that the bunch of granules is an artifact.

In the living egg the two portions of the nucleolus appear as two spherical bodies, but slightly different in texture. Comparison with sections plainly corroborates Flemming's observation that the Nebentheil is more or less swollen by reagents.

## VI. The Centrosome.

In the earliest condition observed, the polar asters, with their minute dark-staining focal granule or centrosome, have already attained their maximum divergence, and their ingrowing rays commence to break through the wall of the germinal vesicle (Pl. XXXIV, Fig. 48).

As in *Thalassema*, a large part of the nuclear skein is thrown out into the cytoplasm, where it undergoes a change in staining power similar to that in *Thalassema ;* but instead of becoming diffused throughout the egg, as in the latter form, it here generally sinks into an irregular black mass on the edge of a vacuole (Pl. XXXIV, Fig. 50).

During metaphase or later, the centrosomes divide (Pl. XXXIV, Fig. 52) and, rapidly diverging, leave in their path a grayish rod-like streak (centrodesmus) which may possibly be the remnant of the cloudy area seen in earlier stages (Pl. XXXIV, Fig. 50). The outer two soon degenerate, while the inner pair, gathering the rays about them in two asters, give rise to the second polar spindle. This, receiving the inner

group of dyads (Pl. XXXIV, Fig. 54), rotates from its paratangential into a radial position, and rises to the surface extremely near the point at which the first polar spindle underwent division.

The divergence of the daughter-chromosomes now follows, during which the inner centrosome does not seem to undergo any noticeable change until telophase, when it divides and appears as two black granules surrounded by a common grayish envelope (Pl. XXXIV, Fig. 53). The rays, while diminishing somewhat in distinctness, still persist with sufficient clearness to enable the aster to be easily recognized as such. During the formation and fusion of the vesicles, the rays still persist and focus to one or two or more granules. In many preparations it is extremely difficult to make sure which of these are the true centrosomes, as the astral center is often somewhat disturbed, whereby other and undoubled cytoplasmic granules become thrust into it. In one very favorable preparation, during the fusing of the vesicles, there are seen at the focus of the persisting egg-aster two black granules, each invested by its own grayish envelope, and exactly similar to the centrosomes of the second polar telophase (Pl. XXXIV, Fig. 56). Little doubt can exist that these are the centrosomes. Remains of the rays are still to be seen after completion of the egg-nucleus, and sometimes the centrosomes show as well (Pl. XXXIV, Fig. 57).

During second polar anaphase and constitution of the egg-nucleus, the sperm-head becomes vesicular and develops an aster (Pl. XXXIV, Fig. 53) focused about a distinct centrosome. An amphiaster, such as Lillie ('97) finds in *Unio*, never arises, and a later division of the centrosome could not be determined with certainty by reason of the numerous cytoplasmic granules surrounding it.

Approach of the germ-nuclei increases the difficulty of making out the behavior of the astral systems, although the indications are that one (either sperm or egg) degenerates, while the other gives rise to the cleavage-amphiaster. This difficulty arises from the fact that renewed activity sets in only after the nuclei have so near approached as to make it impossible to decide to which the centrosomes belong. In some cases the

persistent pair of centrosomes remain beside each other until the germ-nuclei are nearly in contact (Pl. XXXIV, Fig. 58), so that it is here impossible to say which nucleus furnished the centrosomes. In other instances the centrosomes have already considerably diverged when the nuclei approach (Pl. XXXIV, Figs. 59 and 60); but here again the evidence is not satisfactory. Sometimes the centrosomes appear nearer the sperm-, sometimes closer to the egg-nucleus, or equidistant between the two. The close proximity of the centrosomes to the egg-nucleus cannot be taken as a certain indication of their egg origin; for we know that in other forms the sperm-amphiaster may early leave the sperm and wander in toward the egg-nucleus.

From now on the process is clear. With further divergence of the centrosomes, the nuclei meet and incompletely fuse about the developing amphiaster, while *pari passu* the membrane at the poles become more and more pushed into folds by the ingrowing spindle-fibers. The completed spindle lies *between* the two nuclei, so that the chromosomes come to lie in two distinct groups (Pl. XXXIV, Fig. 61).

## VII. The Chromatin.

### a. *In Maturation.*

The demonstration of the chromosomes in the young egg still attached to the wall of the ovary, is not always an easy task. They were observed often enough, however, to dispel all reasonable doubt as to their normal presence and staining capacity in these early stages. They occur in the form of a straight or curled rod, a minute ring, or even a cross. In preparations of unfertilized eggs (sometimes still within the ovary) and early maturation stages, the chromosomes are seen to have undergone considerable growth, and they stand out with great prominence, staining intensely black with haematoxylin. They are situated mostly peripherally along the inner wall of the membrane (Pl. XXXIV, Fig. 47), and have the form of the large double rods, the halves of which may be closely apposed, spread out to form a ring, or disposed in some intermediate

manner, and may be further complicated by becoming curled, coiled, twisted, or bent in a zigzag manner. Their rough and granular composition is quite apparent. Analogy with other forms possessed of ring chromosomes leaves little doubt that these are the product of several transverse and one partial longitudinal division of a spireme thread.

By the concentration of the chromatin, observed so frequently at this period in other forms (e.g., Copepods, Häcker, '95; Insects, vom Rath, '92; Wilcox, '95; *Ophryotrocha*, Korschelt, '95; Selachians, Rückert, '93, etc.), each chromosome entirely loses its granular appearance and becomes converted into a compact tetrad, which consists of four closely apposed loops or spheres (Pl. XXXIV, Figs. 49 and 50).

Along with the rings and rods in the germinal vesicle, cross-shaped chromosomes are also to be met with, and the arms of these may be straight or more or less bent or curved so as to recall the "ophiurid-shaped" chromosomes of Hertwig ('90) (Pl. XXXIV, Figs. 47 and 49). The arms seem never to exceed four in number. In favorable preparations these crosses are seen to be hollow in the center, with indications of a split or seam extending up into two or more of the arms. These chromosomes may in consequence be regarded as rings compressed along two mutually perpendicular diameters, with the four included quadrants thereby converted into loops. Additional evidence is here furnished of the correctness of the interpretation given to the crosses in *Thalassema* (p. 607).

From the open rings it would seem that the tetrad may arise directly by concentration of material at four different points on the circumference. While this may possibly take place sometimes by a looping, such pictures as Pl. XXXIV, Fig. 47, seem to show that the tetrad arises by a curling or tangling up of the ring into a knot at the four points. The result, however, is essentially the same in all cases — a further concentration or contracting of the loops gives rise to a fairly compact tetrad. Upon reaching this stage, or sometimes while still "ophurids," the tetrads commence to enter the forming spindle, and often become thereby temporarily elongated again or variously distorted (Pl. XXXIV, Fig. 49).

When once within the equatorial plate they again assume this compacted condition (Pl. XXXIV, Fig. 50). Often they are not dissimilar to the ring chromosomes in the first spermatocyte division in Elasmobranchs, according to Moore's figures ('95), and if we imagine the latter still further compressed laterally so as to bring the halves into contact, the resemblance would be perfect. Their arrangement within the plate agrees essentially with that of *Thalassema* and is subject to but little, if any, variation (Pl. XXXIV, Fig. 50). The loops are each attached to a single traction-fiber, leaving the lateral ones quite free — an arrangement that occurs as an exception in the first spermatocyte division of *Caloptenus* (Wilcox, '90). Rarely one of the tetrads has adjacent spheres attached to the traction-fibers, as is the rule in *Caloptenus*.

In *Caloptenus* and all forms with similar tetrads the spheres retain their individuality and diverge in pairs. In *Zirphaca*, however, the "spheres," being but contracted loops, the whole chromosome becomes pulled out into a homogeneous ring (Pl. XXXIV, Fig. 51). The quadruple appearance is thus obliterated and is never regained; so that henceforth we have to deal with rings and daughter-V's. The details of the process are easily followed; the elongation of the polar loops (Pl. XXXIV, Fig. 51) and the gradual shortening and disappearance of the lateral loops, followed by division of the ring into daughter-V's (Pl. XXXIV, Fig. 52), as well as the subsequent behavior of the latter, all take place exactly as in *Thalassema*, but with greater clearness.

It is highly probable that the rings and double rods present throughout the growth period arise by a transverse segmentation and partial longitudinal splitting of an original spireme thread, as shown in *Thalassema* and other forms characterized by similar chromatic elements. This being granted, it follows that the polar mitoses represent one "equation" (longitudinal) and one "reducing" (transverse) division. Whether the reduction takes place in the first or the second polar mitosis, is, however, impossible to decide in this case, because of the close similarity in appearance of the four loops.

### b. *In Cleavage.*

In mode of entrance, in appearance and general behavior, the sperm-head of *Zirphaea* perfectly resembles that of *Thalassema*. By second polar telophase the vesicular condition is assumed and the volume enormously increased (Pl. XXXIV, Fig. 53). The intermediate stages in this transformation were not observed. The vesicular sperm-nucleus is completely filled by a rather close chromatic reticulum feebly staining with iron haematoxylin, but deeply with Congo red. It is somewhat pointed in outline, with the sharp end directed toward the centrosome. The egg-nucleus, which soon becomes constituted by the fusion of the vesicles left within the egg after the second polar division, is quite similar in all respects to the sperm-nucleus.

With continued approach, the germ-nuclei increase in size and become perfectly smooth and spherical in outline. Their size is, to all appearance, equal. Meanwhile the chromatin shows a marked increase in its staining power, and just previous to copulation it stains as deeply as the chromosomes (*cf.* Klinckowström, '97, for similar behavior of chromatin in *Prostheceraeus*). The chromatin now exhibits a disposition to arrange in dark-staining strands, the initial stage in spireme-formation. The nuclei have now approached and partially fused to form a bi-lobed segmentation-nucleus. The ingrowing spindle-fibers indicate the commencement of spindle-formation.

In the next stage obtained, the spindle is completed and contains a dense equatorial plate of rod-like chromosomes. Both equatorial (Pl. XXXIV, Fig. 61) and longitudinal sections show these to be arranged in two separate groups that are evidently maternal and paternal, respectively. This agrees with the facts observed by Boveri ('90) in *Pterotrachea;* Häcker ('92); Rückert ('93) in *Cyclops;* Herla ('93) in *Ascaris;* Sobotta ('95) in the mouse; and others that have described a similar independence of the egg- and sperm-chromosomes. By careful orientation of the spindle with reference to the polar bodies, it is seen that the line joining the centers of these chromosomal

groups, which roughly corresponds to that connecting the centers of the copulating germ-nuclei, may be parallel or more or less inclined to the egg-axis.

## PART III.—SUMMARY AND CONCLUSION.

### *Achromatic Structures.*

In respect to gross morphology, the astral systems of *Thalassema* and *Zirphaea* agree closely with Boveri's description of them in *Ascaris*. The minute focal granule may be compared to his "centriole," the cloudy area to his "centrosome," and the centrosphere to the "heller Hof," which with the dense crown of astral rays is equivalent to his "astrosphere."

A detailed study of all stages in their successive order leaves, however, no possible doubt that in the forms I have studied the minute black focal granule ("centriole") is functionally here the true centrosome, as understood by Boveri—the "single permanent cell-organ which forms the dynamic center of the cell and multiplies by division to form the centers of the daughter-cells." It alone of all elements of the astral system *persists throughout all stages*, divides, and apparently initiates mitotic activity. The rays, centrosphere, and cloudy area, which are in turn differentiated about the centrosome, are formed only during the prophases and metaphases. No sooner is the anaphase fairly under way than these begin to break down, while the centrosome, its mission accomplished, migrates to the periphery of the sphere and there sets up a new system often superimposed upon remnants of the old.

By a careful study of these processes, the impression is most strongly conveyed that throughout all these stages the *centrosome is the cause rather than the mere expression or bye-product of the aster-formation.* This is especially clear in late anaphase, where the centrosome deserts the old system and, moving to a different locality, furnishes there the stimulus to the formation of a new one. It is hardly less obvious at the close of the "pause" (during copulation of the germ-nuclei), where after almost complete dying down of the asters the centrosome again furnishes the stimulus to renewed activity.

### *Chromatin.*

In comparing the foregoing description of the chromatin in *Thalassema* and *Zirphaea*, we observe that, while a close parallelism exists between these forms, each complements and throws light upon the other. During the growth period of *Thalassema*, the ring segments of the spireme greatly elongate and, in the full-grown egg, become so distorted that their true form is recognized with difficulty. Sometimes, probably by reason of a temporary loss of staining power, even their presence is hard to demonstrate. Here *Zirphaea* yields important evidence, for the chromosomes are very conspicuous as thick rings in various stages of condensation. During prophase the chromosomes in *Thalassema* again show clearly as open rings or double rods, while in *Zirphaea* the already concentrated tetrads become partially drawn out into rings. In both forms the division takes place by drawing out of the chromosomes into narrow ellipses followed by one cross division whereby V's result. At telophase these break at the angle and do not split longitudinally, as Meves ('95) finds in the Salamander. The process shows most clearly in *Zirphaea*, where the facts demonstrate that the V's cannot arise by bending of a single rod, as Miss Sargant describes in the case of *Lilium*.

The ring or double-rod chromosome is a very common type in the maturation of animal germ-cells, and the rings in most cases undergo a subsequent transformation into quadruple groups or tetrads. The ring is probably the more primitive type, and the four-sphered tetrad probably arises secondarily to facilitate the transference of the chromatin masses during the ensuing divisions. But in all forms heretofore studied, the quadripartite form, when once assumed, is retained and the four quarters distributed among the four daughter-cells. In all forms where a reducing division occurs the elements of the tetrad are so arranged that each sphere comprises exactly one-half of one of the daughter-segments produced by longitudinal splitting of the spireme segments. In *Zirphaea* we find a structure closely simulating an ordinary tetrad, yet the spheres are in this case not homologous with those of a quadruple group. This is

shown by both their origin and their fate; for each sphere is really a loop representing the approximated halves of two adjacent quarters, as shown in the accompanying diagram (III).

In both figures the horizontal and vertical lines represent the division planes, and the included portions (similarly lettered in the figures) homologous parts. The horizontal lines in both coincide with the long axis of the spireme and the transverse axis of the spindle (equation division), while the vertical line

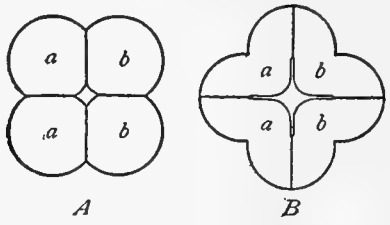



Fig. III.—*A*, tetrad of the Copepod type; *B*, spurious tetrad (cross-form) of *Thalassema* or *Zirphaea*.

is transverse to the spireme-axis or parallel to the spindle-axis (reducing division). In *A* whole spheres are thus separated, in *B* each sphere (loop) is halved.

Should the tetrad shown at *B* be shifted 45 degrees so as to make the division planes separate entire spheres, as is the case in *A*, the formula of the tetrad would be not $\frac{a}{a}\Big|\frac{b}{b}$, as in the first case, but $\frac{\frac{a}{2}+\frac{a}{2}}{\frac{a}{2}+\frac{b}{2}}\Big|\frac{\frac{a}{2}+\frac{b}{2}}{\frac{b}{2}+\frac{b}{2}}$. There seems to be no theoretical reason why such a mode of division may not occur in nature, although up to the present time none has been definitely observed. If the "object" of reducing divisions be, as Weismann supposes, to provide a source of variation, there would be obviously an advantage in the above, since the number of possible combinations is greater in the second case than in the first. The Elasmobranch rings figured by Moore ('95) show four thickenings, which, however, are halved like the loops in *Zirphaea*, but unfortunately, as nothing is said about

the origin of these thickenings, we do not know whether they are homologous with the loops of *Zirphaea* or the spheres of an ordinary tetrad. Bolles Lee ('97) figures and describes a type in *Helix* in which tetrads arise from rings, but later become compacted into solid chromatin masses. His figures suggest the possibility of a rotation whereby the spheres are halved.

It seems not impossible that the mode of division in *Thalassema* and other forms characterized by persistent ring chromosomes may vary in some degree. We have seen that in cases where the ring fails to loop, it may become attached to the fibers in varying ways. If we suppose the attachment to be fixed and to fall anywhere except in the normal division planes, the result will be a division in planes more or less inclined to the normal ones.[1] Weismann ('91) has especially urged this possibility in ring-form chromosomes.

I have found evidence of a type more or less similar to that of *Thalassema* or *Zirphaea* in *Teredo*, *Pholadidea*, and *Nereis*.

Zoölogical Laboratory, Columbia University,
March, 1898.

[1] Persistent ring chromosomes have been described or figured in Plants (Farmer and Moore), Platodes (v. Klinckowström), Elasmobranch (Moore), Mouse (Sobotta).

*All of the figures from camera outlines.*

## EXPLANATION OF PLATE XXXI.

(*Thalassema mellita.*)

FIG. 1. Cluster of minute ova in spireme stage; occasional indications seen of the longitudinal splitting of the chromatin-thread.

FIG. 2. Cluster further advanced; spireme transversely segmented into flattened ellipses; growth period already entered upon.

FIG. 3. Cluster much further advanced; persistence of chromosomes as flattened ellipses within germinal vesicle.

FIG. 4. Cluster of nearly full-grown eggs; persistence of chromosomes; formation of alveoli in meshes of cytoplasm.

FIG. 5. Full-grown unfertilized egg, still in brood-pouch; persistence of chromosomes, rectangular disposition of cytoplasmic strands.

FIG. 6. Egg one minute after fertilization, showing persistent chromosomes and multiple "asters."

FIG. 7. Three minutes after fertilization, showing definitive asters.

FIG. 8. Later stage, asters pushing in wall of germinal vesicle.

FIG. 9. Same stage, showing variation in relation of asters to germinal vesicle.

FIG. 10. Spindle nearly completed, chromosomes in two groups.

FIG. 11. Spindle completed, still central; chromosomes, discarded nuclear reticulum, nucleolus.

FIG. 12. First polar spindle, radially situated, early stage of chromosomes. Sperm-head at ♂.

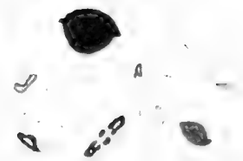
FIG. 13. First polar spindle, showing variations in form of chromosomes.

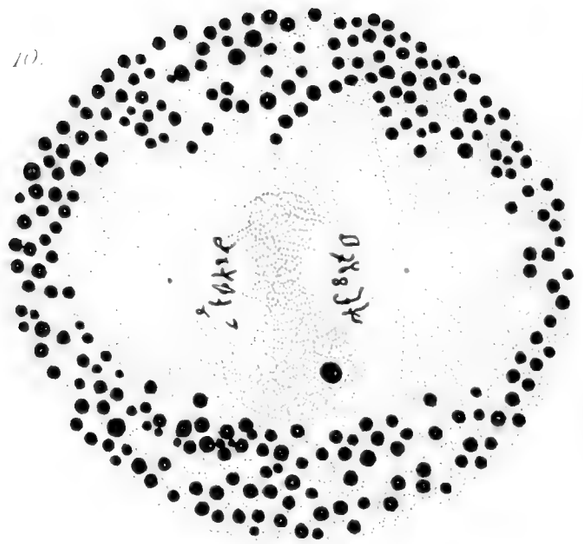
FIG. 14. First polar spindle, showing later stage of chromosomes. Achromatic structures in this and preceding figure entirely schematic.

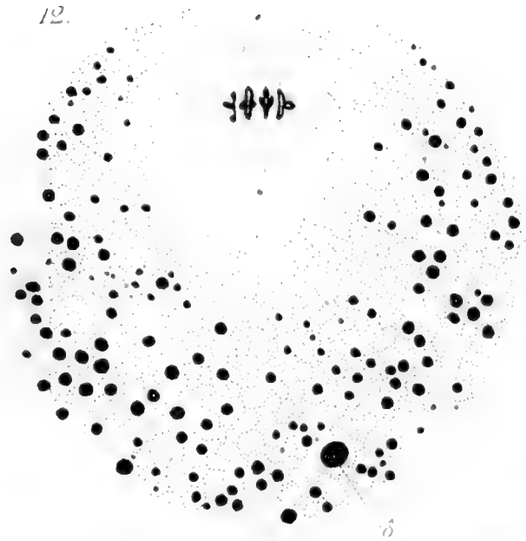
FIG. 15. Anaphase of first polar division; chromosomes diverging as daughter-V's or double rods.

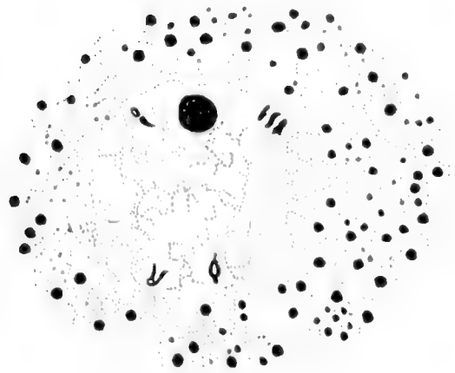
FIGS. 16 and 17. First polar telophase, showing double chromosomes.

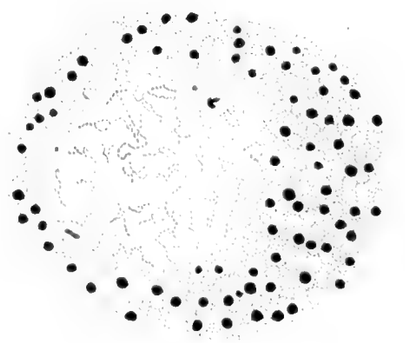
FIG. 18. Second polar amphiaster undergoing rotation.

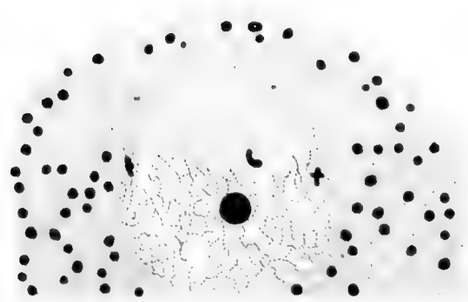


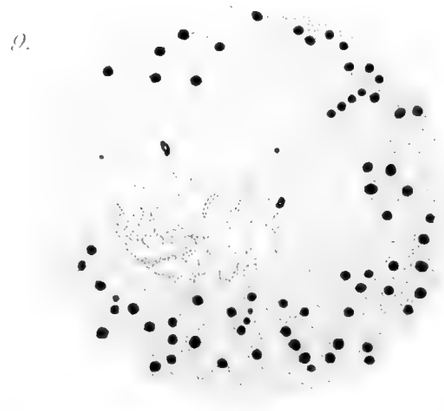












## EXPLANATION OF PLATE XXXII.

(*Thalassema mellita.*)

FIG. 19. First polar telophase, showing inner centrosomes already diverged, in preparation for formation of second polar amphiaster. Sperm-head and aster at ♂.

FIG. 20. Second polar spindle radially situated. Prophase of mitosis of first polar body.

FIG. 21. Same, mitosis of first polar body further advanced.

FIGS. 22–24. Stages in mitosis of first polar body.

FIG. 25. Beginning of second polar anaphase; spindle in polar body situated vertically.

FIG. 26. Second polar telophase; extruding polar body, "cell-plate," double inner centrosome. Sperm-nucleus and amphiaster at ♂.

FIG. 27. Sperm-head with amphiaster.

FIG. 28. Anaphase of mitosis of first polar body. Egg-chromosomes formed into vesicles.

FIG. 29. Polar bodies; abortive attempt at division of the first has resulted in formation of two masses of chromatin, one at each pole.

FIGS. 30 and 31. Telophase of mitosis of first polar body.

FIG. 32. Germ-nuclei approaching; persistence of sperm-centrosomes and asters, disappearance of the egg-center.

FIG. 33. Copulation of germ-nuclei, persistence of centrosomes.

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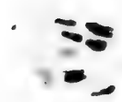

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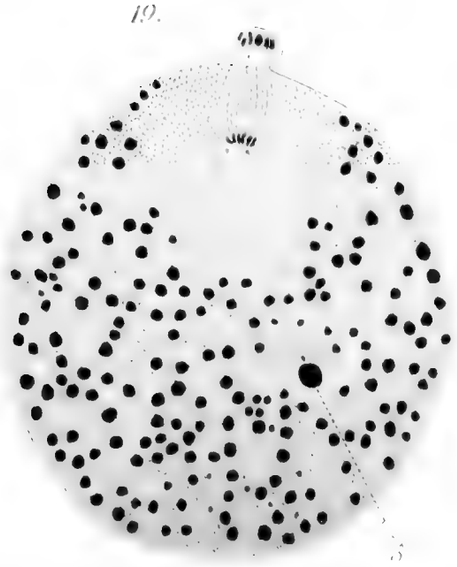

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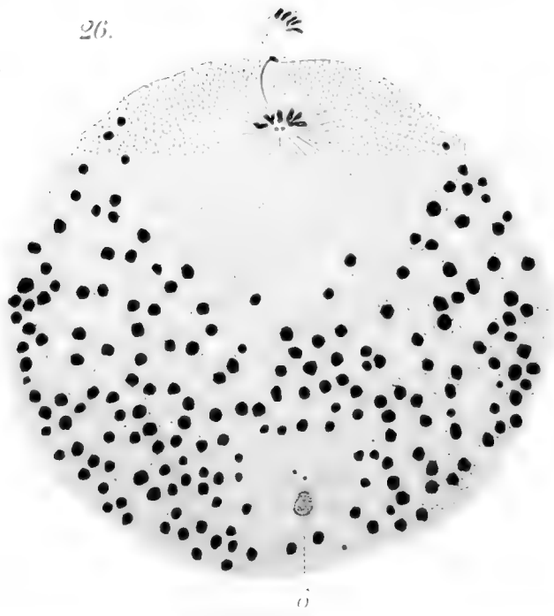

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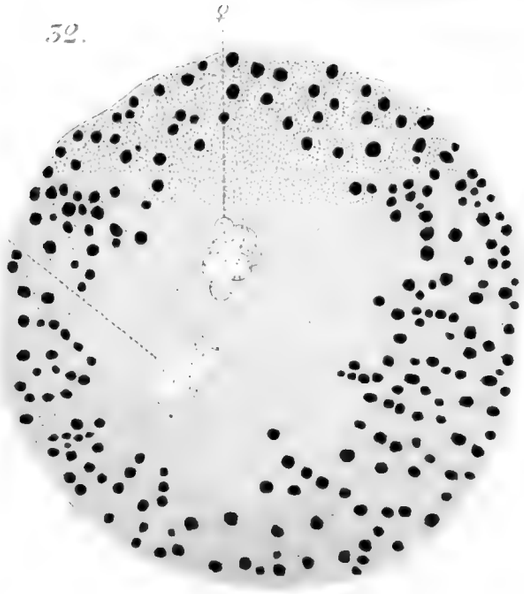

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## EXPLANATION OF PLATE XXXIII.

(*Thalassema mellita.*)

FIGS. 34–36. Various stages in copulation of germ-nuclei, showing persistence of centrosomes throughout entire period.

FIG. 37. Segmentation nucleus; renewed activity on part of centers.

FIG. 38. Later stage, chromatin of nucleus in spireme stage.

FIG. 39. Mitotic figure almost completed; spireme segmenting into the chromosomes; centrosomes divided.

FIG. 40. Transverse section across equatorial plate.

FIG. 41. Beginning of anaphase; centrosomes migrating to periphery of centrosphere.

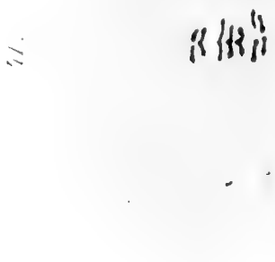
FIG. 42. Anaphase; the centrosomes at the periphery and widely separated.

FIG. 43. Later anaphase; the new amphiaster on the periphery of the former centrosphere.

FIG. 44. Telophase; chromosomal vesicles formed and beginning to fuse; a distinct amphiaster.

FIG. 45. Later stage; nucleus reconstituted.

FIG. 46. Two-cell stage, preparing for the second cleavage; "cell-plate" and engulfed polar bodies between the blastomeres.



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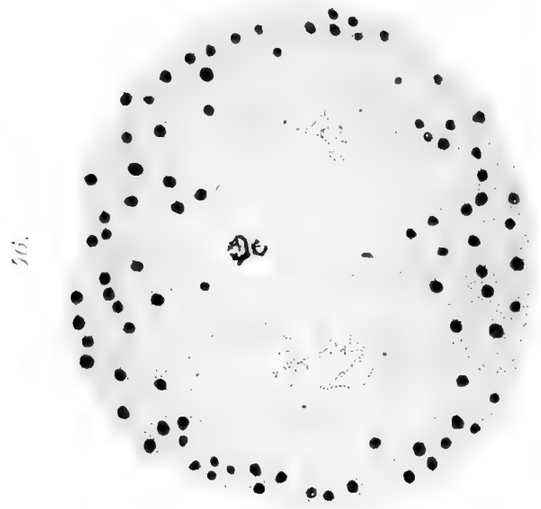

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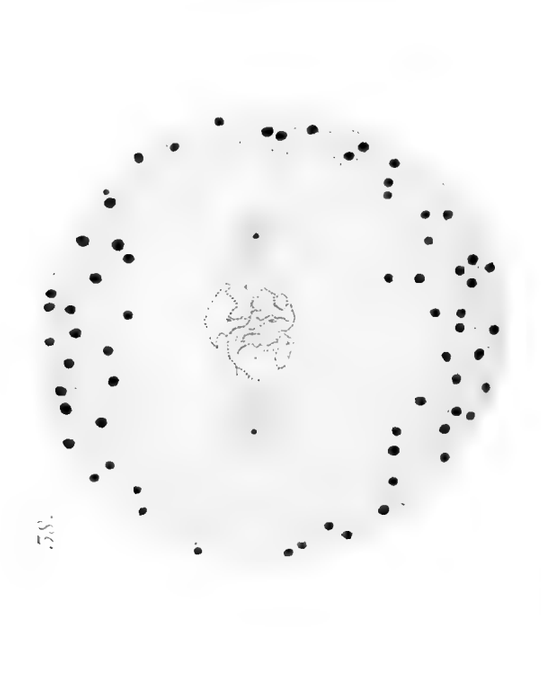

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## EXPLANATION OF PLATE XXXIV.

(*Zirphaea crispata.*)

FIG. 47. Unfertilized egg, showing ring-like chromosomes and double nucleolus.

FIG. 48. Asters pushing in the wall of the germinal vesicle; chromosomes persistent.

FIG. 49. Later stage, with the chromosomes entering the spindle.

FIG. 50. Fully formed first polar amphiaster; the discarded mass of nuclear chromatin at *w*.

FIG. 51. Later stage, the chromosomes pulling out into rings.

FIG. 52. Anaphase; chromosomes diverging as daughter-V's.

FIG. 53. Second polar amphiaster, showing double chromosomes. *1 p. b.*, first polar body.

FIG. 54. Second polar telophase. ♂, sperm-nucleus and aster. *1 p. b.*, first polar body; *2 p. b.*, second polar body.

FIG. 55. Slightly later stage; chromosomes becoming vesicles.

FIG. 56. Approach of sperm-nucleus to the fusing egg-vesicles; persistent egg-centrosomes.

FIG. 57. Egg-nucleus and centrosome.

FIGS. 58–60. Approach of germ-nuclei and divergence of centrosomes.

FIG. 61. Transverse section through equatorial plate of first cleavage-figure, showing separate paternal and maternal chromosome groups.

FIG. 62. Two-cell stage.

FIG. 63. Same, preparing for division.

FIG. 64. Blastula.

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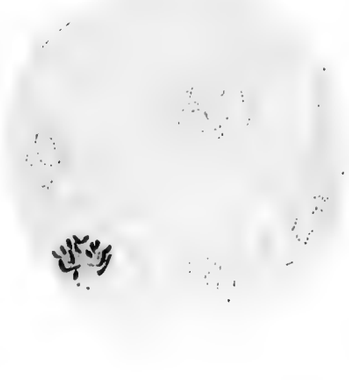

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# ON THE BLOOD-PLATES OF THE HUMAN BLOOD, WITH NOTES ON THE ERYTHROCYTES OF AMPHIUMA AND NECTURUS.

GUSTAV EISEN, PH.D.

CONTENTS.



## *A.* THE BLOOD-PLATES OF THE HUMAN BLOOD.

### I. INTRODUCTORY.

IN a paper on the plasmocytes of *Batrachoseps*, I stated that I had also found plasmocytes in the human blood. At the time of publication of that paper, I had not yet had opportunity to study these structures in the human blood, and could only affirm their presence and suggest that they probably had

been confounded with blood-plates. I believe that I am now able to show that there exist various kinds of blood-plates, and that the true blood-plates in the human blood must be considered as plasmocytes with a complicated internal structure. This is the principal object of this paper. Some observations on the erythrocytes of *Amphiuma* and *Necturus* are appended. For the material of *Necturus*, I am indebted to Prof. William M. Wheeler, of Chicago University, while the *Amphiumas* were procured for me by the California Academy of Sciences, through Messrs. Brimley, of Raleigh, N. C.

## II. Methods of Investigation.

In the preparation of slides, I have found only the dry method to be of any value. The blood is spread in the usual way on cover-glasses, by pulling the latter quickly apart. The glasses must be chemically clean, otherwise the fine filaments of the blood-plates will not be extended. In wiping and polishing the surface of the cover-glasses, a double thickness of soft linen cloth should always be used, as with a single thickness perspiration from the hand will affect the surface. Air-drying for twelve hours, and subsequent fixing with absolute alcohol, are the next two steps in the process, requiring no special description. Fixation by osmic acid and corrosive sublimate-alcohol, as well as with numerous other fixatives, was tried, but found to be injurious. None would give as fine differentiation as the absolute alcohol.

For staining, I found only a few stains of any value. The covers may be most advantageously stained with a weak solution of toluidine in water, for from several minutes to many hours. The best differentiation was had after floating the covers for twenty-four hours in a watch-glass containing a 1 per cent. solution of toluidine. After subsequent washing for a few seconds with distilled water, the covers are quickly dried by the aid of a vaporizer and then mounted in thus-xylol. By this method only blood-plates and leucocytes are stained, and the plates are differentiated very much in the same way as are the plasmocytes in the blood of *Batrachoseps*.

The second method consists in a double staining of eosin and hæmalum. Stain with eosin for five minutes, then wash with water for several minutes, or until no diffuse red stain is visible in the serum-film outside of the red blood-cells. The latter must stand out sharply. Counter stain with a strong undiluted hæmalum for from ten minutes to one-half hour. Wash with distilled water, and mount in thus-xylol. The advantage of this method is that the filaments of the blood-plates are intensely stained red, and can be followed with great facility. The toluidine method stains the filaments less distinctly. The disadvantage of the double staining consists in less differentiation of the inner spheres, as well as in a more or less intense staining of degenerating and broken red cells and other fragments and débris. I have found hæmalum much superior to any of the other hæmatoxylin compounds, in fact the only one which will stain the inner sphere and centrosomes.

The preparations were all studied with a Zeiss Apochromat 3 mm., Aperture 1:40, Ocs. 8 and 12; and with an Abbe achromatic oil immersion substage condenser. The latter is indispensable in order to get a view of the finer details of the blood-plates. In addition I consider it indispensable to use the achromatic light filter, as without it the light will be neither sufficiently pure nor steady enough to differentiate the inner structures of the blood-plates.[1]

## III. Historical Notes on the Nature of the Blood-Plates.

A complete history of the discovery of the blood-plates, and of the various and contradictory views held by investigators in regard to the origin and nature of these bodies, lies outside the scope of this paper, and a few remarks must suffice.

As is well known, the blood-plates in the human blood were first discovered, or at least first described, by Max Schultze, in 1865. He described the outward appearance of the blood-

[1] See *Zeitschrift f. wiss. Mikroskopie u. f. mikroskopische Technik*, Bd. xiv, 1897, pp. 444–447.

plates as they appear under a narrow angle lens and without staining. His figure shows the plates, or, as he calls them, "Plaques" and "Körnchenbildungen," to be simply minute globules of various size, with smooth outlines, although in the text of the paper he mentions that they frequently show fine filaments projecting in all directions, which he contends are nothing but fibrin threads. As to the nature of the "Plaques," Schultze hints at two possible views. Either they may be elementary parts capable of development, or they are simply "Detritusbildungen" or débris. In order not to prejudice any one in favor of the one or the other of these views, he selects for these bodies the name "Körnchenbildungen" as least compromising. Schultze refers also to other granulations in the blood, previously described by Kölliker, H. Müller, Zimmerman, Hensen, Virchow, and others; but I think it of minor importance to decide whether these investigators observed the true blood-plates or simply had before them artifacts or débris.

Bizzozero has, undoubtedly, next after Schultze, contributed most to our knowledge of the blood-plates. In a paper published almost twenty years after Schultze, he describes the blood-plates as a new and independent element of the blood, and one to which he assigns a most important function, — that of causing coagulation of the blood. Since the first researches of Bizzozero several investigators have taken up the study of these minute bodies, especially as regards their origin and functions. The structure and inner organization of the plates have, however, hardly been touched upon, and the discussions have mainly turned on the point of whether the plates are preformed in the blood, or whether they consist simply of chemico-mechanical precipitates or degenerations. To these respective views I can here only briefly refer.

Bizzozero contends that the blood-plates are preformed in the blood, and that they supply material for the fibrin. Hayem, who has studied this subject minutely, not only supposes that the blood-plates, to which he gives the name "hæmatoblasts," are preformed in the blood, but considers them to be erythrocytes, or, in other words, he believes that in time they develop into perfect red blood-corpuscles. His theory has, however,

not found many adherents, as it is readily demonstrated that we do not find in the blood any transition forms between blood-plates and erythrocytes.

On the other hand, a large number of investigators hold that the blood-plates originate from the breaking up of the leucocytes, or that they are in some way altered leucocytes. This view is taken by such biologists as A. Mosso, Ries, Halla, Griesebach, Weigert, Botkin, Lilienfeld, Hlava, and others. Among these, A. Mosso considers the blood-plates to be altered leucocytes, while Lilienfeld and Hlava claim that they consist mainly of nuclein derived from broken-up nuclei, on which account these investigators suggest the name "nuclein-plates."

Hayem, already referred to, must be counted in the above group, as he supposes that the blood-plates originate in the protoplasma of the leucocytes and are projected out from the latter before they enter into the circulation of the blood.

Casimiro Mondino and Luigi Sala compare the blood-plates to the fusiform corpuscles of batrachian, reptilian, and bird blood ; views adopted by nearly all succeeding investigators.

We now come to a large group of investigators, who are of the opinion that the blood-plates are in some way derived from the red corpuscles (the erythrocytes) of the blood. Among these observers we count Klebs, Ponfick, Welte, Bremer, Wlassow, and Prof J. Arnold of Heidelberg, than whom none stand higher, and whose investigations are worthy of the highest consideration. Arnold's earliest investigations were made on blood treated with iodide of potassium, or with normal salt solutions (0.6 per cent.). He found that erythrocytes thus treated disintegrate in various ways, and that the fragments greatly resemble blood-plates. But as through this method only a probability in the result was reached, new investigations were made on living blood in the mesenterium of the mouse, also with the addition of normal salt solution. Arnold thus found that the erythrocytes show minute wart-like or globular elevations or buds, which latter separate themselves from the mother erythrocytes; or some erythrocytes assume a mulberry form, the minute globules of which either separate singly or in mass. The separated globules differ greatly in

regard to the quantity of hæmoglobin contained in them. In other words, Arnold does not hesitate to consider it as definitely settled that the blood-plates are derived directly from the erythrocytes through "Abschnürung" and "Zerschnürung." Arnold also points out that he has found nuclear fragments in the erythrocytes, which fact would explain their supposed presence in the blood-plates. Objections to this theory of the origin of the blood-plates are made by Löwit, Lavdowsky, Scherer, and Wooldridge, who point to the great difference in chemical composition between the erythrocytes and the blood-plates, — differences in hæmoglobin, as well as in staining qualities.

We now mention those according to whose theories the blood-plates are derived from chemico-mechanical precipitations in the blood.

Wooldridge is of the opinion that precipitations of fibrinogen are not to be distinguished from blood-plates ; thus assigning to the latter a chemico-mechanical origin.

Löwit, who has entered into this discussion with much skill and energy, tries to demonstrate that the blood-plates are not preformed in the blood, but that they are partly precipitated from the blood plasma and are partly the débris of leucocytes.

From the above short review it will be seen how different are the views held by the various investigators, hardly any two exactly agreeing as to the origin and nature of the blood-plates. As to the functions of the blood-plates, I believe that most investigators agree that the plates stand in some relationship to the coagulation of the blood. While Bizzozero holds that they furnish the fibrin for coagulation, others, like Ebert and Schimmelbush, contend that they do not form any fibrin but simply conglutinations.

Though many investigators have studied the blood-plates, few have finally agreed upon any points pertaining to their origin, while their structure has hardly been the object of serious research. The summary of our knowledge of the function and origin of the blood-plates is, I think, most clearly contained in the words of Friedenthal (*Biol. Centralblatt*, Bd. xvii, No. 19 (October, 1897), p. 713) : It is with certainty

established through observations under the microscope, that fibrin threads always radiate from heaps of blood-plates; but whether the latter originate from the red or from the white corpuscles of the blood cannot be decided.

In conclusion, I will only state that, according to my own views, the true blood-plates are neither derived from budding erythrocytes, nor from leucocytes, but that they constitute the archosomes — centrosomes with spheres — of erythroblasts or of other nucleated cells, which archosomes have separated themselves from their attachment to the nuclei in the same manner as the plasmocytes of the batrachian blood separate themselves from the fusiform corpuscles.

## IV. Blood-Plates, Plasmocytes, and Fusiform Corpuscles.

As has been mentioned, Bizzozero, Casimiro Mondino, Luigi Sala, and others compare the blood-plates in the human blood to the fusiform corpuscles of some of the cold-blooded vertebrates. Between these respective elements there is, however, a very great difference, both as regards size and structure. The blood-plates in the human blood are many times smaller than the red corpuscles, while the fusiform corpuscles are always of very nearly the same size as the red corpuscles of the same blood. As regards structure, again, we find that the fusiform corpuscles are each characterized by a more or less perfect nucleus, while it can be shown that in the blood-plates no such nucleus exists. The only similarity between the blood-plates and the fusiform corpuscles is that the nature of each causes them to adhere together and to other objects through the aid of peculiar fringed filaments ; thus causing them to form masses of lesser or greater extent.

In the great majority of cold-blooded animals no blood elements are found which can be compared to the blood-plates in the human blood. In the small batrachian, *Batrachoseps attenuatus*, I have, however, recently described minute bodies, exteriorly, which somewhat resemble fusiform corpuscles, but are of smaller size and of a different structure, there being no

nucleus or chromatin present. As will be shown in this paper, these plasmocytes have almost the identical structure of the human blood-plates and must be considered to be of the same nature. I have also shown that these plasmocytes bud out and separate from the fusiform corpuscles, after which the latter decay and disintegrate. It is but a logical conclusion to suppose that the blood-plates of the human blood must have a similar origin ; that they are derived from nucleated corpuscles or erythroblasts, and possess a complicated, distinct, and invariable structure. The blood-plates cannot possibly be considered as precipitations of globulin, fibrinogen, or of other substances in the blood. On the contrary, we must recognize in them a distinct physiological and morphological element of the blood — an element with a phylogenetic life-history and with important physiological functions.

## V. Life-History of a Plasmocyte.

As my original paper on the plasmocytes of the batrachian blood may not be accessible to every one interested in this subject, the following short summary of my former researches may be acceptable. In the blood of batrachians, reptiles, and birds there exist corpuscles void of cell membrane, but furnished with a nucleus. These "fusiform corpuscles," as they are generally termed, are nothing but disintegrating nucleated red corpuscles, which have lost their hæmoglobin as well as their cell membrane. At each opposite pole of this corpuscle is seen at first a very small cytoplasmic projection or bud. In this bud or "plasmocytoblast" we can distinguish the centrosomes of the original red blood-cell, surrounded by several differentiated layers of cytoplasm and archoplasm. This bud grows rapidly, the centrosomes separate from each other, surround themselves with cytoplasmic envelops and show otherwise great activity. At last the bud or buds separate themselves entirely from the nucleus of the fusiform corpuscles and become free and independent elements in the blood. These independent elements I have named "plasmocytes." At first they are very small, but they soon grow and reach even the size of small,

red blood-corpuscles. The cytoplasmic envelops or spheres close up around the centrosomes thus giving the plasmocyte a regular form. Plasmocytes, then, are corpuscles consisting of the original centrosomes surrounded by several cytoplasmic spheres, but not possessing a nucleus. They are the surviving elements of a nucleated erythrocyte, the nucleus and some other parts having disintegrated. The life-history of a plasmocyte can be followed without any difficulty in the blood of *Batrachoseps*, where they are of large size. The blood-plates of the human blood, though very much smaller than the plasmocytes of *Batrachoseps*, possess the same general structure as the latter, and must on this account also possess a similar origin. Within the last few months this plasmocyte theory has been criticised by Dr. E. Giglio-Tos, who contends that the plasmocytes described by me are nothing but altered fusiform corpuscles, and that the granospheres are nothing but disintegrating nuclei. I have no intention of entering into any controversy with Dr. Giglio-Tos upon the merits of his theory, as his criticism is based *exclusively* upon theoretical knowledge and not upon the study of the blood of *Batrachoseps*. An examination of properly prepared material would soon dispel any doubts as to the origin of the plasmocytes, as whole and unbroken series between plasmocytoblasts and plasmocytes are seen on almost every slide, whereas absolutely no connecting links or intermediate stages are found between fusiform corpuscles and plasmocytes ; neither are any to be seen between disintegrating nuclei and plasmocytes ; nor can any possible process of disintegration account for the constant and complicated structure of the plasmocytes. Dr. Giglio-Tos offers his criticism as an "omaggio alla verita scientifica." Would not science have been more honored if the critic, before publishing his conclusions, had actually viewed some plasmocytic blood ; I should certainly have offered him every facility to do so.[1]

[1] Since the above was written, in November, 1897, I have had the pleasure of communicating with Dr. E. Giglio-Tos. After having studied slides of the blood of *Batrachoseps*, as well as the blood of living specimens sent him by me, Dr. Giglio-Tos has changed his views as regards the identity of the plasmocytes with the fusiform corpuscles, or, as he most appropriately calls them, the *trombocytes*. In private letters to me Dr. Giglio-Tos has acknowledged his error in this respect,

## VI. True and False Blood-Plates.

The reason why so much difference of opinion exists in regard to the nature of the plasmocytes or blood-plates must, I think, be sought for in the fact that different structures have been confounded under the common name of blood-plates. In human as well as in batrachian blood, it is quite apparent that the name blood-plate has been given, not only to the human blood-plates described by Bizzozero, which are highly organized bodies, but also to fragments, — disintegrated parts of erythrocytes and leucocytes, — as well as to purely chemical or mechan-

but has not yet expressed any view as to the origin of the plasmocytes. The only published account of his changed views is found on pages 195 and 196 of *I Trombociti degli Itiopsidi e dei Sauropsidi*, Memoria del Dott. Ermanno Giglio-Tos (Accademia Reale delle Scienze di Torino, Anno 1897–98). This is, of course, satisfactory so far as it goes, though I can but think that the proper place for a correction of this kind would have been in the publication where the criticism originally appeared; that is, in the *Anatomischer Anzeiger*. Upon the appearance of the article by Dr. Giglio-Tos, and before I had had the pleasure of corresponding with the Doctor personally, I wrote to the publisher of the *Anatomischer Anzeiger*, asking him to request some one entirely disinterested to study and report upon the microscopic preparations of *Batrachoseps* blood which were sent at the same time. Owing to the very sharp criticism, I thought that this courtesy was due me. The publisher of the journal did not, however, answer my letter.

In connection with this I will refer to the view held by Dr. Giglio-Tos in regard to the origin of the trombocytes. He entirely disagrees with my opinion that the trombocytes are derived from nucleated erythrocytes, and contends that they constitute perfect and independent elements of the blood. Without entering into a full discussion of the subject, I will only call attention to the fact that the nucleus of the trombocyte is invariably found to be in a state of disintegration. Compare the nucleus of the trombocyte with the nucleus of the erythrocyte and with that of the leucocyte and we see at once that it is a degenerating nucleus and not one in a perfect state of preservation. None of the organs or structures of the trombocyte nucleus can be distinguished, no matter what methods are used for fixing or staining. If the trombocyte is a perfect element it must have a perfect structure, and we should at some period find some of these trombocytes possessing a perfectly organized and preserved nucleus. The very beautiful figures accompanying the highly interesting memoir of Dr. Giglio-Tos show the justness of these remarks. Any one who has carefully studied these cells will see at a glance that the nuclei of *all* the trombocytes figured by Dr. Giglio-Tos are in a state of disintegration or degeneration, as far as their morphological structure is concerned. This is the more evident as the author figures, side by side with them, the perfect nuclei of the erythrocytes. (See Figs. 34 to 36, etc.) [Note made at the reading of proof, Oct. 28, 1898.]

ical precipitations of globulin and fibrin. In the blood of the lower vertebrates the fusiform corpuscles have been confounded with plasmocytes or true blood-plates, while the latter have been overlooked. As true blood-plates or plasmocytes, we must consider only those structures possessing a definite, finer, inner organization ; those in which may be distinguished outer spheres forming a cytosome, and inner spheres containing centrosomes, as well as highly refractive secreted or food granules. Such blood-plates are true plasmocytes, partaking of the same origin as the plasmocytes of *Batrachoseps*, having budded off from fusiform corpuscles or from nucleated red blood-cells.

Among the false blood-plates we must distinguish between those which are caused by morphological degeneration and those caused by chemical precipitation. Among the former we must place the fusiform corpuscles which give birth to the plasmocytes ; in the latter class must be included the disintegrating parts of leucocytes, erythrocytes, and nuclei, which are found, respectively, in the blood of the lower as well as in that of the higher vertebrates, including man. Another class of false blood-plates are those caused by purely mechanical and chemical decomposition — precipitations of fibrin and globulin, bodies amorphous as regards shape and without morphological structure. This multiple nature of what has been described as blood-plates has been fully recognized by Friedenthal and Arnold, who both agree that the blood-plates have no unity of origin, but originate by budding and disintegration of red and white corpuscles, as well as from precipitations of fibrin. This assumption is due to a misunderstanding as to the structure of the blood-plates, and cannot be accepted as final. On the contrary, it shows that there must be a distinction made between true and false blood-plates, between blood-plates and débris, between organized and unorganized blood-plates. A diagrammatic view of this would be as follows :

TABLE OF VARIOUS KINDS OF BLOOD-PLATES.

A. *Bodies with Organic Structure.*

1. True blood-plates or plasmocytes, consisting of various spheres with centrosomes and refractive granules. These bodies possess an organization, and the power of growth, movement, secretion, and assimilation, so far found only in mammals and batrachians.

2. Fusiform corpuscles, possessing a nucleus and cytoplasmic spheres. In one species, at least, they give origin to plasmocytes. The nucleus rapidly disintegrates. Found in birds, reptiles, fishes, and batrachians.

3. Disintegrating erythrocytes, leucocytes, and other cells with no special function. Probably found in the blood of all animals.

B. *Bodies without Organic Structure.*

4. Precipitates, chemical or mechanical, of globulin and fibrin. They possess no structure, and must be considered as waste or accidental products.

## VII. GENERAL DESCRIPTION OF THE BLOOD-PLATES.

As I have suggested above, the only elements in the human blood which should be considered as true blood-plates are those small disc-like bodies which possess distinct marginal filaments and show a decided interior organization.

The habit and location of the blood-plates have so frequently been described that detailed reference to them is not necessary. Suffice it to say that they occur singly or in groups, — from a dozen or less up to several hundred, though generally numbering from few to twenty. When in groups, the individual blood-plates may be packed more or less closely together, apparently adhering to each other with their outer fringed edges.

Figs. 1 and 2 illustrate two such groups, stained with eosin-hæmalum. As will be seen, the plates vary considerably both in shape and size. The shape, however, is generally round, or slightly oval, or even irregular, with numerous cytoplasmic filaments. In Fig. 1 some of the blood-plates are seen to be surrounded by a red-staining, homogeneous protoplasm of doubtful nature, while in Fig. 2 very little of such protoplasm is visible, most of the blood-plates being entirely free or isolated.

Many groups of blood-plates are not surrounded by such a plasma, and it can be seen that they adhere to each other exclusively through their cytoplasmic projections. As regards size, the blood-plates differ greatly from each other. Compared with the red corpuscles of the blood, they are of course very much smaller. In a general way it can be said that, taking the groups of blood-plates as they are found, it takes from seven to twenty plates to equal in size a red corpuscle, the varying distance of the plates from each other being taken into account. Some of the blood-plates are four or five diameters larger than others. I have frequently found blood-plates the long diameter of which equaled two-thirds the diameter of a red corpuscle. Generally, however, they are much smaller, and I think it safe to say that it takes about ten of the average size blood-plates to equal a red corpuscle, the surface of the latter alone being considered. Owing to the exceeding minuteness of the blood-plates, their uncertain boundaries, and filamentous, cytoplasmic projections, as well as to their variability in size, direct measurements would be difficult to make and unreliable.

## VIII. Detailed Description of the Blood-Plates.

Even a superficial examination of a blood-plate shows that it consists of three distinct envelops or zones, one interior to the other, and more or less concentrically arranged.

*A.* In or near the center we find usually one, sometimes two (seldom more), very bright, highly refractive globules, of roundish, compact, irregular or regular form, and of a white or yellowish color. They appear like a diamond in a dark setting. These highly refractive globules do not stain.

*B.* Surrounding these highly refractive bodies is seen a darker area containing one or more dark granules, the latter generally situated close together and connected by a dark-staining film or irregular zone.

*C.* Exterior to this is seen an outer, lighter-staining zone, with many filamentous, cytoplasmic projections, by which the blood-plates are made to adhere to each other and to other objects in the blood.

If we now examine these respective zones more in detail, we find that they show characteristics of great constancy. The inner, highly refractive bodies are nearly always well defined, with a very sharp outline. In many are seen concentric layers, though in the majority none such can be recognized, probably on account of the minuteness of the objects. The refractive power is always very great and very striking. These granules vary greatly in size, some being barely perceptible (Pl. XXXV, Figs. 4, 13); others are so large as to occupy a large part of the blood-plate (Pl. XXXV, Figs. 5, 12, 17). The inner, dark-staining mass surrounding these refractive bodies is differentiated into several parts, all of which cannot, however, always be shown to be present at the same time. In the center of the mass, generally in close proximity to the refractive body, is seen a darker zone with from one to three or even more granules of a yet darker color. This darker zone may be either small or large, sometimes occupying the larger part of the blood-plate (Pl. XXXV, Fig. 17); at other times it is confined to the vicinity of the refractive body (Pl. XXXV, Fig. 18).

In some blood-plates (Pl. XXXV, Figs. 6, 12, 13, 16, etc.) the central dark zone contains one or more light areas surrounding the darker granules. The minuteness of the blood-plates is such that these, the smallest of the interior structures, lie near the limit of vision, and only the most delicate manipulation of the light will show them with any degree of satisfaction. There is also a great difference between the individual blood-plates, some being successfully stained, others not. But even in those less differentiated enough can be seen to show that there exists a very remarkable and constant differentiation as regards structure.

We now come to the outer envelop or zone of the blood-plate. In the toluidine preparations this zone is very faintly stained and barely visible, and the fine filaments can be followed only in isolated instances. With the eosin-hæmalum preparations the case is different; here the filaments are distinctly stained, sometimes even intensely brought into view, but there is less differentiation of detail. In the blood-plate stained with toluidine we can often see a slight differentiation of this outer

zone into an inner and outer layer, the inner one being much lighter in color. The outer layer sends out a number of cytoplasmic projections and filaments, which frequently are longer than the diameter of the main body of the blood-plate.

Recapitulating, we find that the blood-plate contains the following zones or spheres, more or less concentrically arranged. Counting the innermost first we have:

1. A highly refractive, unstainable granule (food granule or ferment).
2. A darker-staining zone, generally containing darker granules (somosphere with centrosomes).
3. A lighter, irregular zone (centrosphere).
4. A darker, granulated zone (granosphere).
5. A lighter, unstainable zone, not always distinguishable (hyalosphere).
6. An outer, fringed zone (plasmosphere), stainable with eosin.

## IX. Blood-Plates and Plasmocytes.

From the above description it becomes apparent that the blood-plates of the human blood show a very great similarity to the plasmocytes of the blood of *Batrachoseps*. In many instances, indeed, the structure is identical even to the minutest details. A comparison of the various figures given in this paper with Pl. II, Figs. 57, 60, 80, 81, etc., of my former paper ('97), shows this similarity to be so great as to leave no doubt as to the indentical nature of the blood-plates and the plasmocytes.

The prominent, inner, refractive body or bodies in the blood-plates are also seen in many of the plasmocytes. I have suggested that these refractive bodies may constitute food granules or secreted matter, and I think they are entirely distinct from the centrosphere (*l. c.*, p. 10). Among the dark-staining structures of the blood-plates we meet with zones corresponding to the centrosomes, somosphere, centrosphere, and granosphere of the plasmocytes; but the minuteness of the blood-plates is such as to make a segregation of these zones most difficult, even under favorable circumstances, and in most instances it is impossible. There is, however, no doubt but that the inner dark-staining granules, which frequently occur in groups of three, must be identified as centrosomes, having the same nature, appearance, and staining qualities as the centrosomes in the

plasmocytes. The somosphere and centrosphere in the blood-plates are less apparent, though in many instances (Pl. XXXV, Figs. 13, 16) I have satisfied myself of their identity. The large, dark-staining, granulated area, which is always present in the blood-plates, I do not hesitate to identify with the granosphere of the plasmocytes of *Batrachoseps*. It is large enough to be readily seen and recognized. The outer filiferous zone of the blood-plates so greatly resembles the two outer zones of the plasmocytes that their identity must be apparent even after the most superficial examination. The filaments of the blood-plates are longer than those of the plasmosphere of the plasmocytes, but their nature is otherwise the same. Through the aid of these cytoplasmic projections, the plasmocytes as well as the blood-plates adhere both to each other and to other objects in the blood. On account of this great similarity in structure, as well as in outward appearance and behavior, I do not hesitate to say that the true blood-plates in the human blood are plasmocytes, and, as such, must have the same origin as the plasmocytes in the blood of *Batrachoseps;* that they are really composed of the centrosomes, with several cytoplasmic spheres originally budded off from some nucleated red cells in the human body, probably the erythroblasts in the bone marrow. The process of separation has not been studied, but it is safe to presume that it must, at least in a general way, be similar to that observed in the blood of *Batrachoseps* (*l. c.*, p. 18, etc.).

## X. Functions of the Blood-Plates or Plasmocytes.

According to Bizzozero and others, the blood-plates in the human blood, as well as the fusiform corpuscles in the blood of the lower vertebrates, must be considered as being the direct cause of coagulation of the blood. They are great, or perhaps even exclusive, producers of fibrin. That the blood-plates act as repairers of injuries to blood-vessels is now hardly ever denied by investigators. The principal difference of opinion arises in the question as to whether this function of the blood-plates is not also shared by leucocytes and perhaps even by red blood-corpuscles. To this controversy I cannot add any opinion based on ex-

tended investigations, but can only point out a few observations which I believe have not been recorded by others. In my former paper on the plasmocytes, attention has been called to the fact that these plasmocytes actually engulf and digest food, such as bacteria and débris of erythrocytes and other cells, and that they frequently possess in their interior one or more highly refractive granules, which it is suggested may be food granules, stored for future use. The plasmocytes of the human blood are too minute to allow of a detailed investigation of any such engulfed food particles, but still they are sufficiently large to enable us to clearly distinguish the presence of highly refractive granules of the same nature as those in the plasmocytes of *Batrachoseps*, and similarly located as regards the various spheres.[1]

In coagulated blood and in thrombosis the finer structure of the blood-plates appears to be greatly confused, and so far I have not been able to devise any method by which this structure can be satisfactorily studied under these conditions. I have found in coagulated blood many highly refractive granules scattered in the serum, but am not certain that they can be considered identical with the highly refractive granules of the blood-plates and plasmocytes. The similarity is sufficiently great, however, to suggest that possibly these granules are not really food granules stored for future use, but that they are secreted granules, perhaps a ferment causing the coagulation of the blood when ejected from their place in the plasmocytes into the blood serum.

The highly organized structure of the blood-plates indicates that they cannot, as has been supposed by many, be originated in the blood with great rapidity and precipitated at the very moment that they are required. The blood-plates must possess a phylogenetic life cycle, through which they have acquired structure, increased by growth, accumulated food, and secreted certain highly refractive matter. In a word, the blood-plates must be considered an independent element of the blood, of equal rank with the red and white corpuscles, and of hardly less importance.

[1] Since the above was written I have repeatedly found in the human blood blood-plates which contained débris of erythrocytes.

## *B.* THE BLOOD-CORPUSCLES OF AMPHIUMA AND NECTURUS.

### XI. GENERAL REMARKS ON THE ERYTHROCYTES.

In the following pages I will confine myself to a description of the structure of the red corpuscles and to their derivatives, the fusiform corpuscles. I expect to show that in the blood of the above-named batrachians we must distinguish between two different kinds of red blood-corpuscles, differing from each other in size, form, and structure. In one class (Pl. XXXVI, Figs. 22, 23) we find groups of centrosomes suspended free at the poles of the cell, far away from the nucleus; while in the other class (Pl. XXXVI, Fig. 24) we find no such groups, but, on the contrary, an archoplasmal structure with centrosomes, situated in a granosphere close to the nucleus. Corresponding to the two classes of red corpuscles we find two classes of fusiform corpuscles derived from the former. In one class the centrosomes are seen to be free and far away from the nucleus (Pl. XXXVI, Fig. 25); while in the other the archoplasmal structures lie in a depression of the nucleus, one at each pole. The structure of the centrosomes will be described in detail; and as regards their nature and function in the first-mentioned class of corpuscles, it may be suggested that their function is purely mechanical, for the purpose of balancing and guiding the large oblong cells through the capillaries, thus preventing stagnation and undue obstruction.

### XII. TWO KINDS OF ERYTHROCYTES AND TWO KINDS OF FUSIFORM CORPUSCLES.

Not only in the blood of *Amphiuma* and *Necturus*, but in all other batrachian and reptilian blood examined by me, have been found two distinct kinds of nucleated erythrocytes. One is more round than the other, and the two kinds stain somewhat differently. In *Amphiuma* and *Necturus* erythrocytes of the oblong kind described above possess directing globules, while the rounder kind have none. The nucleus of the former is longer and narrower, with an uneven outline; that of the

latter is broader, with an even, smooth outline. There is also another important difference between the two cells. The nucleus of the rounder cell generally possesses a slight depression at each pole, and adjacent to this is seen a narrow, differently staining zone, which under certain favorable conditions resembles the granosphere with centrosomes in the fusiform corpuscles. No such zone is found in the longer corpuscles. It appears, therefore, as if the groups of globules in the longer corpuscles are morphologically of the same nature as the centrosomal spheres in the round corpuscles.

As might be expected, we also find in the blood of these species two distinct kinds of fusiform corpuscles. One kind (Pl. XXXVI, Fig. 25) is undoubtedly a degenerated oblong red corpuscle, retaining all its characteristics. The nucleus is in a far advanced state of degeneration, while the groups of globules and granules are yet in position at the poles. The other kind of fusiform corpuscle is shorter. The nucleus possesses a depression at each pole, and outside of this we find the cytospheres, granosphere, centrosphere, and centrosomes, more or less distinctly brought out. These latter structures correspond to the plasmocytoblasts in the blood of *Batrachoseps*. In the blood of *Necturus* and *Amphiuma* they do not develop into plasmocytes.

## XIII. Structure of the Oblong Erythrocytes.

The oblong red corpuscles of both *Amphiuma* and *Necturus* are so similar that in the following pages I shall refer to them together, after first having mentioned the difference between them. Even with a comparatively low power it will be seen that at each pole, outside of the nucleus, there exist one or more groups of dark-staining granules. In *Necturus* there is generally only one such group at each pole, while in *Amphiuma* there are several, nearly always arranged in pairs of two, four, or six, at or near the pole. Occasionally we find smaller groups at the sides, or even isolated globules. In Figs. 22 and 23 I have figured two erythrocytes from *Amphiuma*, and in Fig. 29 one from *Necturus;* both under a moderate power

of magnification. In *Necturus* the groups are always smaller than in *Amphiuma*, but otherwise there is a great resemblance between them.

Under the highest systems these groups resolve themselves into a number of small globules of different sizes and shapes; and in the majority of globules we find one or more small, dark granules. The appearance of these globules and their darker granules is such that I can only compare them to centrosomes surrounded by a differentiated and differently staining sphere. As to the nature of this sphere I am somewhat undecided, but from analogy am inclined to consider it identical with the somosphere, previously described (Eisen, p. 17, etc.). Especially in *Necturus* is it readily demonstrated that these globules are surrounded by or connected by a very thin, foam-like cytoplasm of irregular form. Whether this is cytoplasm pure and simple, or whether it partakes of the nature of archoplasm in the sense of Boveri, is not to be determined at present, as the hæmoglobin in the cells evidently prevents a proper differentiation. The somospheres and centrosomes are, however, readily stainable, and a study of them is not connected with difficulties, provided double staining is not attempted. Every effort at double staining with eosin or with fuchsin mixtures has proved a failure. With such stains these groups are not even brought out, but remain unstained. They stain readily, however, with basic aniline dyes, such as the methylen blues, toluidines, thionins, gentian-violet, etc. I have found polychromes-methylen-blue one of the best. With this latter stain we find that the globules are not always as regular as they appear to be at first. They are sometimes confluent, sometimes send out ramifications and projections, as, for instance, in Pl. XXXVI, Figs. 27, 28, etc. As a rule, we find one or more dark granules in each such projection or globule, but in many instances there are none to be seen. The globules may be more or less numerous in each group. In *Necturus* there are seldom more than a dozen, while in *Amphiuma* I have sometimes counted thirty in a group. The darker granules appear to increase in number by budding in a manner similar to that of centrosomes, as described by Heidenhain, and this

is my principal reason for identifying them as such. These granules are sometimes well defined, sometimes with blurred outlines, which latter, however, may be ascribed to imperfection in staining, due perhaps to the surrounding hæmoglobin.

It is possible that these globules with granules are of a nature similar to those described by Ludwig Bremer from the red blood-cells of *Testudo carolina*. There are, however, some very great differences in structure and shape, due perhaps in part to the methods employed by Bremer, whose preparations were principally fixed with osmic acid. When I employed his method, I was not able to bring out any of the finer structures of these groups, and I am satisfied that osmic acid, as well as most other fixatives used, instead of fixing, distorts the structure of the blood-cells. But there are some characteristics in the paranuclei of Bremer which are not found in the cells now considered. His paranuclei are less regularly situated and often appear to be connected with the true nucleus. I have at times found structures similar to Bremer's in *Diemyctylus torosus*, in which species I have, however, never seen any globules similar to those in *Necturus* and *Amphiuma*. Taking all in all, it seems as if the globules of these genera are different from the paranuclei of *Testudo;* or, if of the same origin, they must possess a different function.

## XIV. Function of the Globules.

In none of my preparations did I succeed in finding any erythrocytes in mitosis, and therefore cannot decide whether the globules and granules have any function connected with cell-division. I doubt their having any such function, as their great number would rather tend to disarrange karyokinesis than to assist it. Any other function that may be ascribed to them is probably of a purely mechanical nature. How this is possible will, I think, be more clear when we remember, as I have before pointed out, that there are two kinds of red blood-corpuscles, one of which is rounder than the other and does not possess the numerous globules of the longer kind. The unusual length of the red cells carrying the globules is most striking.

Such great length must be in certain respects a disadvantage to the free circulation of the cells, which if turned sideways must necessarily be impeded in their travel. If, however, the poles could be loaded and increased in weight, the cells would be more apt to travel with their pointed ends forward, and their speed would thus be increased instead of retarded. I believe the function of the globules is to thus load the poles of the cells and keep their pointed ends forward instead of sideways. This opinion is strengthened by the fact that the rounder erythrocytes possess no such directing globules.

## XV. SUMMARY.

1. We must distinguish between true and false blood-plates, and between organized and non-organized blood-plates. The only true blood-plates are those of plasmocytic nature. The false blood-plates are either fusiform corpuscles, degenerating fragments of leucocytes and erythrocytes, or chemico-mechanical precipitations of fibrin and globulin.

2. In the blood of *Batrachoseps* the organized blood-plates are of two kinds, fusiform corpuscles and plasmocytes, the latter derived from the former. In the blood of many other batrachians and reptilia the organized blood-plates are only fusiform corpuscles, no plasmocytes being found.

3. In the human blood the true blood-plates are true plasmocytes, possessing the same general nature and structure as those in the blood of *Batrachoseps*. In these blood-plates in the human blood we may distinguish various zones and spheres. These are :

*a*. Three outer cytoplasmic spheres : cytosphere, hyalosphere, and granosphere.

*b*. Three inner spheres : centrosphere, somosphere, and centrosomes.

*c*. A centrally or laterally situated, highly refractive granule, which may be either a food granule, or a secreted product, perhaps a ferment causing the coagulation of the blood. All the true blood-plates of the human blood have this structure.

4. In the human blood, under certain conditions, there may also be found false blood-plates, caused by precipitation of glob-

ulin, degenerating cells, etc. ; but these plates do not possess any constant and differentiated structure, nor any definite and constant form. In healthy blood all the blood-plates are true plasmocytes, and even in diseased blood the plasmocytes constitute the only true blood-plates.

5. For true blood-plates I propose to drop the word plates and substitute the word *plasmocytes*.

6. In batrachian and reptilian, as well as in bird's blood, we must recognize two distinct kinds of red blood-corpuscles (erythrocytes) and two kinds of fusiform corpuscles. Each kind of red blood-cell degenerates into a distinct kind of fusiform corpuscles.

7. In *Amphiuma* and *Necturus* one class of erythrocytes is oblong, the other more rounded. They possess, respectively, the following characteristics :

*a*. Oblong erythrocytes.

Quite oblong ; nucleus oblong without depressions at the poles, with uneven or warty outline. At the poles of the cells, far from the nucleus, are found paired or single groups of granules and globules, probably identical with centrosomes and granosphere, and surrounded by a thin archoplasm.

*b*. Rounded erythrocytes.

More rounded, some entirely round ; nucleus rounded, with smooth outline. Generally a depression at each pole, furnished with the various spheres characteristic of a plasmocytoblast. There are no separate globules at the poles.

8. The two kinds of fusiform corpuscles partake of the same general characteristics. They have been derived from the erythrocytes through degeneration and are generally not possessed of a cell membrane. In the round fusiform corpuscles the plasmocytoblasts are active, shown by increasing size ; but in *Necturus*, *Amphiuma*, *Diemyctylus*, *Chondrotus*, *Plethodon*, and many others they do not develop into plasmocytes.

9. The function of the globules and granules (centrosomes with somospheres) in the oblong erythrocytes is probably a mechanical one. It consists of so loading the poles as to cause them to be directed forward, while the erythrocytes travel through the capillaries. The advantage of such an arrange-

ment is to prevent a blocking of the capillaries and to cause an increase of speed in the travel of the long erythrocytes through them. The rounder erythrocytes do not require this loading of the poles, as their shape would not interfere with their movements in the capillaries.

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## EXPLANATION OF THE FIGURES.

FIGS. 1, 2, 17, 18 are from preparations stained with eosin-hæmalum. Figs. 3, 16, 19, 21, stained with toluidine. Figs. 23–27, metanil-yellow; polychromes-methylen-blue. Fig. 20, the same as the last, but with after treatment of weak oxalic acid in water. Figs. 28–36, metanil-yellow and polychromes-methylen-blue. Thus-xylol. All were studies with Zeiss, Apo. 3 mm., Apt. 1 : 40, Ocs. 8 and 12. Figs. 1, 2, 22, 23, 26, 27, 30, 33, 36, projection on table. All the other figures are drawn to a larger scale, in order to be more distinct. Figs. 22–24, 30 drawn from Zeiss D., Oc. 4.

The only light used was that passed through the light filter previously described; daylight being very unsatisfactory.

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## EXPLANATION OF PLATE XXXV.

*Plasmocytes, or Blood-Plates, from Human Blood.*

FIG. 1. A group of plasmocytes, partly surrounded by free protoplasm of doubtful nature. Some of the plasmocytes are free, all possess cytoplasmic projections. The white centers are highly refractive, unstainable granules. The dark field surrounding them contains the archosomal zones, such as centrosomes, centrosphere, etc. The red-colored parts contain the cytoplasmic spheres, with longer or shorter cytoplasmic filaments.

FIG. 2. Another group of plasmocytes, or blood-plates, of the human blood. The plasmocytes are adhering to each other, but there is no diffuse protoplasm. The details are as in Fig. 1.

FIG. 3. A free plasmocyte. In the two outer spheres we can recognize the plasmosphere and the unstained hyalosphere. The granosphere is stained dark violet. The centrosphere is white, and the centrosomes, with presumably a somosphere, are stained dark.

FIG. 4. A plasmocyte with two separate groups of centrosomes, between them a paler centrosphere.

FIG. 5. A plasmocyte with a large refractive granule, a paler centrosphere with centrosome. Another centrosome lies outside of the granosphere, and outside the latter are the other cytoplasmic spheres, not separable from each other.

FIG. 6. A plasmocyte with four central centrosomes and three centrospheres, one of which contains a centrosome.

FIG. 7. A plasmocyte with two centrosomes, surrounded by a food, or ferment, granule. The dark zone is the granosphere; the two outer paler zones, the cytoplasmic spheres.

FIG. 8. A plasmocyte with a very large central granule, at the edge of which is seen a centrosome. The granosphere is dark; the outer cytoplasmic spheres are pale blue.

FIG. 9. A group of plasmocytes of different sizes illustrating the variation in form and size. The largest plasmocyte with two groups of centrosomes, three of

which are in each group. The two round, pale bodies are probably food, or secreted, granules of a highly refractive nature. The central paler body is perhaps the centrosphere. In *b* the large body is the granule, also a single centrosome in a centrosphere. *c* possesses a single centrosome, and *d* two groups of centrosomes, one of which is less defined. The plasmocyte (*a*) has a diameter about one-half that of a red corpuscle.

FIG. 10. A plasmocyte with three centrosomes placed inside the granule.

FIG. 11. A plasmocyte with two refractive granules and three free centrosomes in a group. The fourth centrosome is surrounded by a centrosphere.

FIG. 12. A large plasmocyte which appears as if in division. The large white fields are refractive granules. The centrosomes are surrounded by a centrosphere.

FIG. 13. One of the largest plasmocytes observed in human blood. Its diameter equals about two-thirds that of a red blood-corpuscle. There are three groups, each with several centrosomes and a granule. The very large, paler field is probably a centrosphere, in the center of which is a somosphere with a single centrosome. There are two smaller plasmocytes, possibly offshoots of the same group.

FIG. 14. A plasmocyte with two granules and a centrosphere with a single centrosome.

FIG. 15. A plasmocyte with a large granule and a centrosome and centrosphere lying independently in the granosphere.

FIG. 16. A plasmocyte similar to the former, but with a group containing three centrosomes.

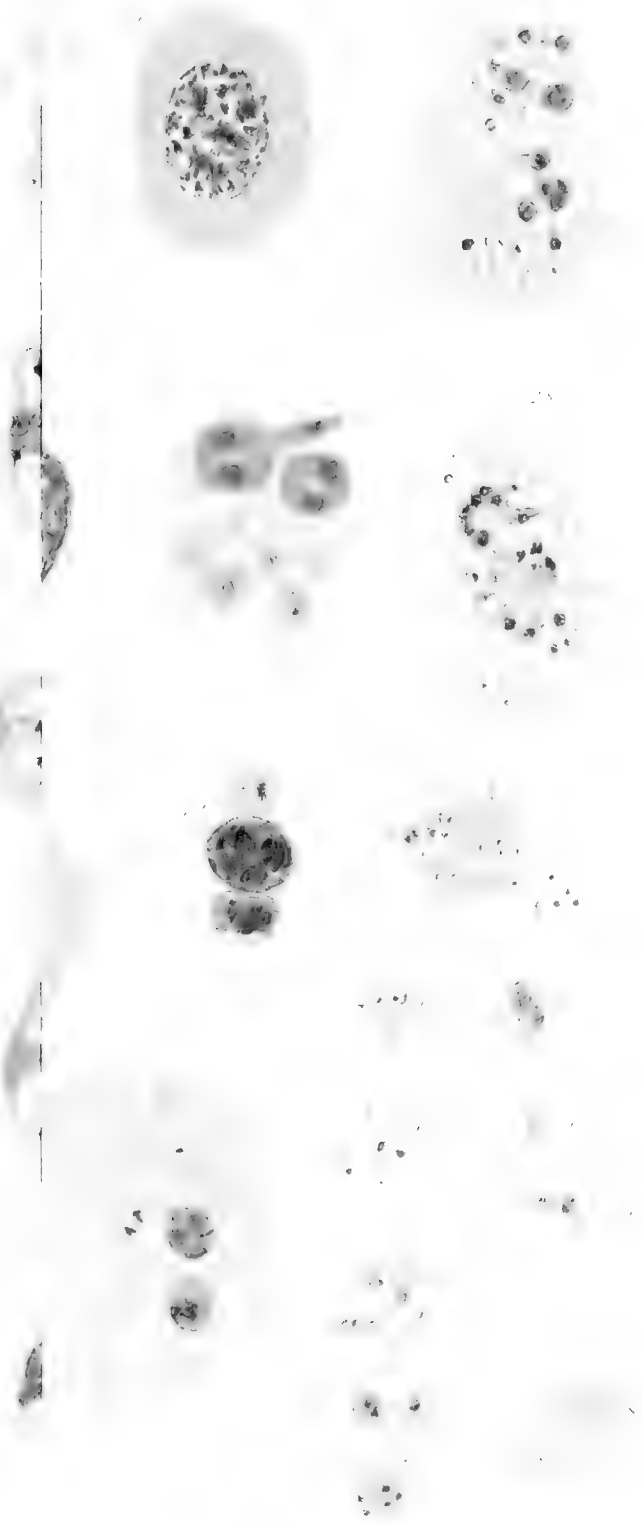
FIG. 17. A plasmocyte with a very large, highly refractive granule, surrounded by three darkly stained centrosomes, which again are surrounded by a granosphere.

FIG. 18. A large plasmocyte similarly organized to the last one. The centrosomes appear to be connected by a somosphere. The large granulated red area is the granosphere. This, as well as the last plasmocyte, is from a slide stained with eosin-hæmalum, which stain has brought out more definitely the cytoplasmic filaments.

FIG. 19. A plasmocyte with a large lower refractive granule, an upper centrosphere, and two centrally situated centrosomes.

FIG. 20. A plasmocyte with true, distinct centrosomes and a large granule. Whether the darker areas surrounding the centrosomes are to be explained as somospheres is uncertain.

FIG. 21. In this plasmocyte we have two large granules of a highly refractive nature, with a single centrosome between them.



Eisen, del.

B. Meisel, Lith. Boston

Figs 1-21 Plasmocytes Human Blood. Figs 22-28 Bloodcorpuscles etc., of Amphiuma mæans. Figs 29-36 Bloodcorpuscles etc., from Necturus.

## EXPLANATION OF PLATE XXXVI.

*Amphiuma mæans.*

FIG. 22. An erythrocyte or red blood-cell. Zeiss D., Oc. 4. A large central nucleus and four separate groups of centrosomes situated at the poles. These groups are of about the same size, and equidistant.

FIG. 23. Another erythrocyte, in which the four groups of centrosomes are less equal in size, and not as regularly distributed; still each group is quite distinct. This and the former figure represent the most common class of red corpuscles, in which the centrosomes are situated far away from the nucleus and grouped at the poles of the cells.

FIG. 24. A red blood-corpuscle, or erythrocyte, belonging to a different class from the last two. The cytoplasm in this class stains with basic stains. There are no groups of centrosomes at the poles as in the former class, but close to the nucleus is seen a pale, unstained zone, undoubtedly containing centrosomes and spheres. These latter are only brought to view in the fusiform corpuscles.

FIG. 25. A fusiform corpuscle derived from an erythrocyte, or red blood-corpuscle, of the class shown in Figs. 22 and 23. At the poles are seen centrosomes. The nucleus is in strong degeneration, or dissolution. The hæmaglobin is already diffused, and the outer membrane of the cell is breaking up. This class of fusiform corpuscles is entirely distinct from the following.

FIG. 26. A fusiform corpuscle of the other type, derived from a red corpuscle of the kind shown in Fig. 24. The nucleus is degenerated, but not to such a degree as the one represented in Fig. 25. At each pole is seen a zone with differentiated spheres and centrosomes, corresponding to the plasmocytoblasts of the fusiform corpuscles of *Batrachoseps*. In this instance, however, they do not develop into plasmocytes. The paler outer zone corresponds to the plasmosphere and hyalosphere in the plasmocytes.

FIG. 27. Two groups of centrosomes from a pole of a red blood-corpuscle of *Amphiuma*. One or more centrosomes are seen to be surrounded by a protoplasmic envelop, which probably can be considered homologous to a somosphere.

FIG. 28. Another group of centrosomes with somospheres. In many of the smaller somospheres no centrosomes are visible. The group appears to be in active division and development.

## EXPLANATION OF PLATE XXXVII.

*Necturus maculatus.*

FIG. 29. A red blood-cell, or erythrocyte, under a low magnification, showing the groups of centrosomes at each pole. The following figures show either entire groups of centrosomes more highly magnified, or isolated centrosomes with somospheres. As will be seen in the red blood-cell of *Necturus*, there is only one single group of centrosomes at each pole, while in the red blood-cells of *Amphiuma* there are generally two or more.

FIG. 30. A group of centrosomes. Some of the isolated bodies I consider to be centrosomes surrounded by a somosphere. The pale yellowish zone surrounding these bodies in Figs. 27, 28, 30, and 36 may be common cytoplasma, or perhaps a centrosphere. In Fig. 30 it is seen to have a distinct foam structure. Each one of the two upper globular somospheres contains two centrosomes. The one to the left has sent out a bud, in which is seen a rather undefinable centrosome, perhaps an offspring from one of the larger ones.

FIG. 31. Another group of centrosomes. The large somospheres are rounded, globular, oblong, or even ring-like. In most of them are darker bodies, which I consider to be identical with the centrosomes of Heidenhain, or the centriols of Boveri. In many somospheres no centrosomes are found.

FIG. 32. A somosphere sending out two buds, one of which is globular, the other oblong. The dark bodies are the centrosomes. Many of these are not well defined in the drawings, not being very distinctly outlined on the preparations, which probably was due to optical or other imperfections.

FIG. 33. A whole group of centrosomes and somospheres from the pole of one of the red blood-corpuscles.

FIG. 34. Another group of somospheres and centrosomes more highly magnified. Oc. 18.

Fig. 35. A group of centrosomes, with somospheres from one of the poles of a red blood-corpuscle. Some of the somospheres apparently possess no centrosomes. Oc. 12.

FIG. 36. *a–h* represents five whole groups of somospheres and centrosomes from the poles of the red blood-corpuscles. Figs. *e*, *f*, *g* represent isolated somospheres with darker stained centrosomes. In the other figures the yellow ground-substance represents either pure cytoplasm or centrospheres.

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## STAINS.

*Polychromes-methylen-blue*, nach *Unna*. Dr. G. Grübler & Co., Leipzig.

*Eosin*. James W. Queen & Co., Philadelphia, U.S.A. Already mixed and in solution, composition unknown.

*Toluidine blue*, extra. Actien-Gesellschaft f. Anilin-fabrication, Berlin (66,711), 1 per cent. watery solution, 10 per cent. alcohol.

*Metanil-yellow*. Actien-Gesellschaft f. Anilin-fabrication, Berlin.

All stains were supplied by C. C. Riedy, San Francisco, Cal.

# THE PHOSPHORESCENT ORGANS IN THE TOAD-FISH, PORICHTHYS NOTATUS GIRARD.

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Assistant Professor of Physiology, Leland Stanford Junior University.

CONTENTS.



## I. Introduction.

Fishes described as possessing phosphorescent organs belong almost without exception to the deep-sea fauna. They live at depths where the light of the sun rarely if ever penetrates, and this fact is supposed to account for the process of evolution which has brought about the phylogenetic development of light-producing organs. It lent interest, therefore, to the study of phosphorescent organs in fishes when in 1889 a paper appeared, describing phosphorescent organs in Porichthys, a shore fish. The study of these organs was made on unfavorable material, as the author states, and as the structures described were so obviously different from phosphorescent organs in other fishes, it seemed desirable to reinvestigate the question.

It is my purpose in this paper to present the distribution, structure, and development of the phosphorescent organs in Porichthys, together with their surface relations to the lateral-line system of sense organs.[1] In the future I hope to follow with a second paper on the morphology of the lateral-line system, work now partially completed.

The results presented in this paper were obtained chiefly from the study of Porichthys notatus Girard. But I have also some material from Porichthys nautopedium Jordan and a new species of Porichthys kindly furnished me by Prof. C. H. Gilbert.

Porichthys notatus is found abundantly along the Pacific coast from Sitka to Panama. It is taken in early spring and summer at tide water where it comes to spawn. The eggs are cemented in a single layer to the under surfaces of stones, and, as the male remains with the brood until the young become free-swimming (when they are about one inch in length), it is comparatively easy to secure adults together with young. Adults are also taken in large numbers in the trawls on the fishing banks north of San Francisco in spring and summer. In winter they are very scarce on the banks.

In a surface view, the phosphorescent organs appear as bright silvery spots distributed in lines or rows over the surface of the body of the fish. The average number of organs is about 350 on each side of the fish, 700 organs in all. Of these, 275 on either side are located in eight lines on the ventral and ventro-lateral surface of the body. In this region the rows are very conspicuous (Pl. XXXVIII, Figs. 1 and 2), but more obscure

[1] The material for this study was collected and the work begun at the Hopkins Seaside Laboratory, Pacific Grove, Cal., in the summers of 1892 and 1894. I am deeply indebted to the directors, Dr. O. P. Jenkins and Dr. C. H. Gilbert, for the privileges of the laboratory and for advice and encouragement. I wish also to express my indebtedness to Dr. C. O. Whitman for the privileges of a table at the Marine Biological Station, Woods Holl, Mass., during the summers of 1896 and 1897; also to thank the members of the instructing corps for suggestive criticism and advice. Miss Clapp has kindly allowed me to examine her manuscript on the Lateral-Line System of Opsanus tau, and has criticised my manuscript, as well as given me many suggestions as to methods of making preparations. And, finally, I am especially indebted to my wife, whose invaluable criticism and enthusiastic interest have been my constant support.

or sometimes entirely absent over the dorsal aspect of the body. Each organ, viewed from the surface, appears as a silvery spot more or less circular in outline and varying in size from a point scarcely visible with the unaided eye to .8 mm. in diameter. The larger organs are found on the ventral surface of the fish, the less conspicuous ones on the dorsal surface — a fact to be again referred to later in the paper. They are often surrounded by, or bordered on one side by, an increased amount of pigment. This is especially noticeable in organs on the sides of the body where the pigment is much increased in amount on the side of the organ away from the mid-ventral line.

The structures, indiscriminately designated by Test as "lateral-line organs," "slime glands," "mucous pores," or "pores," are in reality lateral-line sense organs of the kind designated as nerve hillocks by Merkel. Such an organ, viewed from the surface, presents a point on the epidermis free from pigment, the end of the sense organ itself with its immediately surrounding supporting cells. It is too distinct and characteristic in appearance to be mistaken by even the most casual observation.

The lines of phosphorescent organs and lateral-line organs are so closely associated in their distribution in the skin of the fish that they may be most economically mapped out together. Test did not discriminate between these two sets of organs. Terms he introduced, referring to lines of organs, will be indicated by quotation.

## II. Distribution of the Phosphorescent Organs and the Lateral-Line Sense Organs.

The organs of both systems are arranged in lines or rows and are very constant in their relation to each other in any given row, also the rows are constant in their relative positions in different individual fishes. The number of organs in a given line of either phosphorescent organs or sense organs may, however, vary much in different individuals (see Table). The average number only will in most instances be given in the general description.

The lateral-line organs are located free on the surface with the exception of certain rows on the head which are in canals. These lines of canal organs form only a small part of the lateral-line system in Porichthys. Unless otherwise specified, surface organs are meant.

It is impossible to determine the homologies of the parts of the complex lateral-line system without a knowledge of the development of the sensory Anlage and of the innervation of the system, including the origin of the nerves in the central nervous system. Such a study has been made in part for only a few fishes, while the great mass of fishes remain unknown in this regard. It is necessary, therefore, while awaiting the development of our knowledge of the origin and distribution of the lateral-line nerves, to designate the groups of lateral-line organs in particular species by some sort of descriptive terms. The intention in this paper is not so much to name the groups in Porichthys as to describe their location by short descriptive terms that will serve temporarily, *i.e.*, until we have a surer foundation for establishing homologies between the parts of the lateral-line system in different species of fishes, when a permanent nomenclature may be adopted.

### 1. *Lines of Organs on the Body.*

The lateral row, *la* (Pl. XXXVIII, Figs. 1–3), begins on the side at a point posterior to the upper border of the pectoral fin and directly below the third dorsal ray. It runs straight back along the side to the upper third of the base of the caudal, and contains both kinds of organs. This line contains an average of thirty-six sense organs with a phosphorescent organ immediately below and generally one above each sense organ. In the lower series the phosphorescent organs are well developed and large, but the organs of the upper series are quite small and rudimentary; in fact they are often wanting, there being only an average of twenty-two in the fifteen specimens tabulated. The organs in this line correspond very nearly with the segments of the part of the body along which they lie. They are found above the grooves which mark the boundary between the myomeres.

The "pleural" row, *pl*, consists of parallel lines of phosphorescent organs and sense organs. The row of phosphorescent organs begins at a point posterior to the middle of the base of the pectoral and below the anterior ray of the dorsal fin. The line curves backward and downward to a point back of the lower edge of the pectoral and above the first anal ray, thence straight back along the side, ending usually above the twenty-third anal ray. The organs of this line vary in number from forty-three to sixty-two, or an average of fifty-three, and have no relation to the body segments. The row of sense organs is located immediately below the row of phosphorescent organs and follows the same general course. This row usually extends backward only to the thirteenth anal ray, but in three specimens from Alaska (see Table, Nos. 8–10) the row extends to the base of the caudal fin. There is an average of thirty-two sense organs in the row, excluding the three exceptions mentioned. In these three there are sixty, bringing the general average up to thirty-six.

There are two caudal, *ca*, lines of sense organs on each side the fin, located on the upper and lower thirds, respectively. These rows contain only sense organs, which are well developed at the base of the caudal, but become smaller toward the extremity of the fin. There are as many as twenty-five in each line in the oldest specimens, but the number varies greatly, increasing with the age of the specimen. These rows are in line with the lateral and pleural rows of sense organs, but they are not continuous with them.

The "anal" row, *a*, runs along the body on either side of the base of the anal fin from opposite the interspace between the second and third anal rays to the base of the caudal. The phosphorescent organs of this line correspond in position to the anal rays and are in pairs, with exceptions to be mentioned. The first organ is usually single; the next twenty-eight or twenty-nine, paired; the last six or seven, single. The last four or five organs are situated under the base of the caudal and are arranged in a markedly compact row. There is an average of thirty-six organs in this row, counting the pairs but once. The outer organs of the pairs are apparently larger than

the inner, but this appearance is in part due to the fact that the inner organs are more deeply buried in the angle of the base of the fin. The posterior two or three pairs are closely united. The anal row contains a single line of sense organs. There are thirty-four of these organs in the line. The first is placed just in front of the second pair of phosphorescent organs, and each successive one bears the same relation to its corresponding phosphorescent organ, except the last two, which are placed external to and just above the four or five phosphorescent organs along the base of the caudal.

The "ventral rows, *v*, form a parenthesis on the stomach," extending from the side of the anus three-fourths the distance to the ventral fin. The anterior ends of the two rows are usually continuous with each other, and comprise thirty-four phosphorescent organs on either side. The organs present a clear, circular outline without apparent increase of pigment around them. These rows are not accompanied by sense organs.

The "gastric" line of phosphorescent organs, *ga*, begins a little below the middle of the front of the base of the pectoral, curves forward, downward, then backward, around the lower edge of the pectoral; then extends straight back along the side of the belly to a point dorsal to the anterior edge of the anal papilla. The line contains an average of thirty organs, about ten in the curved portion and twenty in the straight part of the line.

The "gular" line, *gu*, begins a little back of the isthmus and runs parallel with its fellow backward to the posterior and ventral side of the ventral fin, then curves outward and backward to a point below the anterior end of the straight portion of the gastric line. There is an average of twenty-seven organs in this row. The gular line gives off a short spur of seven organs running forward along the external border of the ventral fin.

The gastro-gular line, *ga gu*, of sense organs begins at the isthmus somewhat anterior to the end and toward the median ventral side of the phosphorescent row, runs posteriorly parallel to the phosphorescent line to its end, then backward parallel to

the gastric row, curves upward around its posterior end, and terminates in close relation to the pleural row of sense organs (see Pl. XXXVIII, Fig. 1). There are fifty organs in the entire row.

The "scapular" row, *sc*, begins just back of the posterior pore of the temporal canal, runs straight back above the pectoral fin, then curves in toward the base of the dorsal fin, where it is continued into the dorsal row at the base of the third dorsal ray. There are seventeen sense organs in this row, with an average of ten phosphorescent organs alternating with them.

The straight part of the scapular row is accompanied by a scapular accessory row, *sc ac*, of three to five sense organs, with a small phosphorescent organ above each.

The dorsal row, *d*, extends along the dorsal surface of the body at the base of the dorsal fin from the third dorsal ray to the caudal peduncle. This row contains an average of seventy-one sense organs. Rarely phosphorescent organs are found between the first three or four organs of the row, but in such exceptional specimens they are always small and rudimentary.

Outside the dorsal row is an irregular line, or accessory row, *d ac*, which contains sense organs and rudimentary phosphorescent organs arranged as in the lateral line. The number of organs in this row is quite variable, an average of sixteen in five specimens (see Table).

### 2. *Organs of the Lower Jaw and Head.*

The branchiostegal row, *br*, begins in front of the isthmus and extends outward over the membrane of the gill-cover to the base of the lower branchiostegal ray ; then along the membrane between the first and second rays almost to the edge of the gill-cover. There are thirty-four phosphorescent organs in this line and no sense organs.

The "mandibular" row, *md*, of phosphorescent organs extends around the inner rim of the ridge formed by the dentary bones. It contains twenty-two organs on either side.

The operculo-mandibular row consists of surface organs and canal organs. It begins on the side of the head at the anterior pore of the temporal canal. It runs downward on the surface

to in front of the opercular spine, where it sinks into a canal. This canal extends along the posterior border of the preopercle, around the angle of the mouth, and forward on the ventral surface of the mandible to near its anterior end, where the line comes again to the surface and ends at the symphysis of the jaw (Pl. XXXVIII, Fig. 2). There are eight pores or openings to the canal portion. This line contains twenty-one organs, seven at the upper free end, seven in the canal, and seven at the lower free end. At the upper free end there are occasionally rudimentary phosphorescent organs between the sense organs — an average of one.

There are two "opercular" rows, an upper and a lower. The upper row, *u op*, contains ten phosphorescent organs and fourteen sense organs. These are arranged in parallel lines, the phosphorescent organs above and the sense organs immediately below them. The line begins posterior to the second pore from above of the operculo-mandibular canal, and extends in a curve backward and upward, over the operculum, to a point posterior to the opercular spine. The row of sense organs is the longer of the two.

The lower row, *l op*, contains from two to ten phosphorescent organs, on an average four, and an average of seventeen sense organs, the phosphorescent organs occurring between the sense organs at the base of the line. The row begins opposite the third operculo-mandibular pore and extends backward and upward to near the posterior angle of the opercular flap. The curvature of the lower row is a little greater than that of the upper, the two ending near each other.

The infraorbital consists of two portions, a preorbital and a suborbital, each of sense organs. The preorbital or nasal portion is made up of a row of four to six organs from the posterior nasal opening to the base of the anterior nasal papilla, two pairs of organs between the anterior nasal papillae, and two to three more in a transverse line at the posterior base of the tube. The suborbital extends from the posterior nasal opening around under the eye to the anterior pore of the temporal canal. It contains fourteen sense organs and one large phosphorescent organ below the posterior border of the eye.

The temporal canal (Pl. XXXVIII, Fig. 3, *t*) corresponds to the squamosal of Allis in Amia. It contains a single canal organ. Above this canal are two free organs. The infraorbital, temporal, scapular, and dorsal form a continuous series of sense organs.

A short maxillary canal, *mx*, with two organs, extends along the maxillary bone below the posterior nasal opening. At its lower end are two free organs with a small phosphorescent organ above each in very old specimens.

A malar row of sense organs, *ma*, extends downward from the suborbital across the cheek to the angle of the mouth, thence along the mandible over the operculo-mandibular canal to its anterior end. There are thirty-one organs in this row, thirteen in the malar portion and eighteen in the mandibular. Two spurs are connected with this line, a short anterior spur of two organs from the middle of the cheek portion of the line and a second posterior one of four to five organs from the angle formed at the mouth.

The supraorbital line, *sup o*, is enclosed in a canal which begins by a pore at the median border of the posterior nasal opening, runs backward and inward toward the median line of the head, where it anastomoses with its fellow of the opposite side at a point in a transverse line drawn through the posterior border of the lens of the eye. Here the canals diverge and each runs to a point behind the eye a distance equal to half the diameter of the latter. This canal opens at its ends only. Five sense organs are found in the canal — three in the anterior part and two in the posterior limb.

The "frontal" group, *fr*, consists of two rows of organs. It is located over the posterior end of the frontal bone, about midway from the median line of the head to the outer edge of its flat top. The outer row of each group consists of an average of six sense organs, with five phosphorescent organs, alternating in a longitudinal row. The inner row is a shorter one, parallel with the posterior part of the outer row, and consists of from four to five sense organs with phosphorescent organs at the inner and outer edge of each sense organ. The phosphorescent organs of this group, like those of other dorsal groups, are especially variable in number and rudimentary.

The "occipital" row, *oc*, contains from nine to twelve sense organs, sometimes with a pair of rudimentary phosphorescent organs on the inner and outer side of each sense organ, sometimes with no phosphorescent organs. The row begins near the posterior edge of the spinous dorsal, curves first inward toward the median dorsal line, then outward and forward (see Pl. XXXVIII, Fig. 1).

In a species described from the Galapagos Islands, Porichthys nautopedium Jordan, the arrangement of phosphorescent organs and sense organs corresponds, group for group, with that given above. There are only slight variations from the average number of organs, except in the pleural row, which, like the pleural row of three specimens from Alaska (Table, Nos. 8–10), is continued back to the base of the caudal.

In the new species of Porichthys previously referred to, obtained recently at Panama by Dr. Gilbert, the location of the lines of phosphorescent organs and sense organs corresponds very closely with that in Porichthys notatus. Also the number of both kinds of organs, as will be seen by a reference to the appended table, Nos. 16 and 17, is very similar to the number in the common form. It may be noted, however, that on the dorsal surface, where in Porichthys notatus rudimentary organs are found, phosphorescent organs are wholly absent. The phosphorescent organs on the ventral surface of this species are not more than half as large as in the common species.

In general, we may say that the phosphorescent organs of the three species of Porichthys studied are always well developed and prominent along the ventral and ventro-lateral surfaces of the body, while along the dorsal surface they are markedly small and rudimentary, and are very variable in their development in the different specimens of the same species.

The sense organs, on the other hand, are quite constant both in their presence and the extent of their development in the different regions. The sense organs are accompanied by dermal papillae, two for each organ. These dermal papillae differ very much in the extent of their development, being most

southern Alaska coast. Specimens 16 and 17,

| Temporal. S.O. | Maxillary. S.O. | | Malar. Ph. | Malar. S.O. | Supra-orbital. S.O. | Outer Frontal. Ph. | Outer Frontal. S.O. | Inner Frontal. Ph. | Inner Frontal. S.O. | Inner Frontal. Ph. | Occipital, Ph. | Occipital, S.O. | Occipital, Ph. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2+ | — | — | — | — | 5 | 5 | 5 | 5 | 5 | 4 | 9 | 4 |
| 1 | 2+ | — | — | 27 | — | 7 | 9 | 3 | 5 | — | 0 | 10 | — |
| 1 | 2+ | — | — | — | — | 6 | 7 | 3 | 5 | 2 | 4 | 9 | 4 |
| 1 | 2+ | — | 4 | 33 | — | 5 | 6 | — | 4 | 2 | 1 | 9 | 3 |
| 1 | 2+ | — | — | 29 | — | 5 | 6 | 1 | 4 | 4 | 0 | 10 | — |
| 1 | 2+ | — | — | 25 | — | 5 | 6 | 1 | 3 | 1 | 3 | 11 | 1 |
| 1 | 2+ | — | 4 | 26 | — | 5 | 5 | 4 | 4 | — | 2 | 8 | 3 |
| 1 | 2+ | — | — | — | — | 7 | 7 | 5 | 5 | 3 | 12 | 12 | 12 |
| 1 | 2+ | — | — | — | — | 7 | 7 | — | 3 | — | — | 9 | — |
| 1 | 2+ | — | — | — | — | 6 | 6 | 5 | 7 | 5 | — | 12 | — |
| 1 | 2+ | — | 4 | 35 | — | 4 | 7 | 4 | 4 | 4 | — | 9 | — |
| 1 | 2+ | — | 0 | 34 | — | — | — | — | — | — | 0 | 9 | 0 |
| 1 | 2+ | 2 | 2 | 35 | 5 | 6 | 6 | 5 | 5 | 5 | 0 | 8 | 0 |
| 1 | 2+ | 2 | 5 | 25 | 5 | 0 | 6 | 2 | 4 | 2 | 0 | 10 | 0 |
| 1 | 2+ | 2 | 0 | 37 | 5 | 7 | 7 | 3 | 5 | 3 | 0 | 12 | 4 |
| 1 | 2+ | 2 | 3 | 31 | 5 | 5 | 6 | 3 | 4 | 2 | 2 | 10 | 2 |
| 1 | — | — | 0— | 25— | 5 | 0— | 5— | 0— | 3— | 0— | 0— | 8— | 0— |
| | | | 5 | 37 | | 7 | 9 | 5 | 7 | 5 | 12 | 12 | 12 |
| 1 | — | — | — | — | — | 0 | 10 | 0 | 5 | 0 | — | — | — |
| 1 | — | — | 0 | 25 | — | 0 | 8 | 0 | 7 | 0 | 0 | 11 | 0 |

TABLE SHOWING VARIATIONS IN THE NUMBER OF PHOSPHORESCENT ORGANS AND OF LATERAL-LINE SENSE ORGANS.

Specimens 1 to 15, Porichthys notatus. Numbers 1 to 7 and 11 to 15, inclusive, off the California coast. Numbers 8 to 10, off the southern Alaska coast. Specimens 16 and 17, Porichthys, sp. indsc., from Panama.

| No. | Lateral. Ph. | Lateral. S.O. | Lateral. Ph. | Pleural. Ph. | Pleural. S.O. | Caudal. Upper. S.O. | Caudal. Lower. S.O. | Anal. Ph. | Anal. S.O. | Ventral. Ph. | Gastric. Ph. | Gular. Ph. | Gastro Gular. S.O. | Scapular. Ph. | Scapular. S.O. | Scapular Accessory. Ph. | Scapular Accessory. S.O. | Dorsal. Ph. | Dorsal. S.O. | Dorsal Accessory. Ph. | Dorsal Accessory. S.O. | Dorsal Accessory. Ph. | Branchiostegal. Ph. | Mandibular. Ph. | Operculo-Mandibular. Ph. | Operculo-Mandibular. S.O. | Upper Opercular. Ph. | Upper Opercular. S.O. | Lower Opercular. Ph. | Lower Opercular. S.O. | Preorbital. S.O. | Suborbital. Ph. | Suborbital. S.O. | Temporal. S.O. | Maxillary. S.O. | Malar. Ph. | Malar. S.O. | Supraorbital. S.O. | Outer Frontal. Ph. | Outer Frontal. S.O. | Inner Frontal. Ph. | Inner Frontal. S.O. | Inner Frontal. Ph. | Occipital. Ph. | Occipital. S.O. | Occipital. Ph. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 26 | 34 | 34 | 62 | 28 | — | — | 35 | 32 | 32 | 30 | 31 | 48 | — | 16 | — | — | 3 | 70 | — | — | — | 31 | 20 | — | —+7+ | — | 8 | 14 | 2 | 16 | — | 1 | 16 | 1 | 2+ | — | — | — | — | 5 | 5 | 5 | 5 | 5 | 4 | 9 | 4 |
| 2 | 32 | 35 | 35 | 56 | 35 | — | — | 34 | 32 | 39 | 29 | 36 | 51 | 12 | 17 | — | — | 0 | 63 | — | — | — | 33 | 22 | — | 8+7+ | — | 11 | 14 | — | — | 9 | 1 | 14 | 1 | 2+ | — | — | 27 | — | 7 | 9 | 3 | 5 | — | 0 | 10 | — |
| 3 | 24 | 35 | 35 | 52 | 34 | — | — | 36 | 33 | 32 | 29 | 32 | 49 | 7 | 17 | — | — | 0 | 79 | — | — | — | 36 | 18 | — | 7+7+ | — | 10 | 15 | 3 | 16 | — | 1 | 16 | 1 | 2+ | — | — | — | — | 6 | 7 | 3 | 5 | 2 | 4 | 9 | 4 |
| 4 | 23 | 35 | 35 | 57 | 37 | — | — | 37 | 34 | 31 | 32 | 34 | 49 | 10 | 20 | — | — | 0 | 73 | — | — | — | 34 | 23 | 4 | 10+7+ | — | 11 | 15 | 3 | 19 | 9 | 1 | 16 | 1 | 2+ | — | 4 | 33 | — | 5 | 6 | — | 4 | 2 | 1 | 9 | 3 |
| 5 | 25 | 31 | 31 | 46 | 19 | — | — | 36 | 34 | 27 | 31 | 34 | 45 | 9 | 17 | — | — | 0 | 66 | — | — | — | 35 | 20 | — | 6+7+ | — | 8 | 12 | — | 13 | 9 | 1 | 16 | 1 | 2+ | — | — | 29 | — | 5 | 6 | 1 | 4 | 4 | 0 | 10 | — |
| 6 | 25 | 36 | 34 | 53 | 34 | — | — | 36 | 34 | 27 | 31 | 33 | 47 | 13 | 16 | — | — | 0 | 72 | — | — | — | 32 | 21 | — | 7+7+ | — | 9 | 12 | 4 | 15 | 8 | 1 | 15 | 1 | 2+ | — | — | 25 | — | 5 | 6 | 1 | 3 | 1 | 3 | 11 | 1 |
| 7 | 31 | 35 | 35 | 51 | 29 | — | — | 36 | 33 | 26 | 28 | 33 | 44 | 12 | 17 | — | — | 5 | 68 | — | — | — | 34 | 20 | 4 | 7+7+ | — | 12 | 14 | 5 | 15 | 10 | 1 | 15 | 1 | 2+ | — | 4 | 26 | — | 5 | 5 | 4 | 4 | — | 2 | 8 | 3 |
| 8 | 28 | 37 | 37 | 47 | 64 | — | — | 38 | 36 | 32 | 30 | 38 | 53 | 11 | 20 | — | — | 2 | 72 | — | — | — | 36 | 22 | — | 9+7+ | — | 12 | 14 | 5 | 18 | 7 | 1 | 18 | 1 | 2+ | — | — | — | — | 7 | 7 | 5 | 5 | 3 | 12 | 12 | 12 |
| 9 | 7 | 38 | 38 | 55 | 62 | — | — | 40 | 36 | 36 | 31 | 36 | 59 | 13 | 19 | — | — | 0 | 75 | — | — | — | 35 | 23 | — | 7+7+ | — | 11 | 15 | 4 | 19 | 8 | 1 | 17 | 1 | 2+ | — | — | — | — | 7 | 7 | — | 3 | — | — | 9 | — |
| 10 | 14 | 36 | 36 | 43 | 58 | — | — | 39 | 37 | 32 | 27 | 34 | 52 | 7 | 18 | — | — | 0 | 75 | — | — | — | 37 | 21 | — | 6+7+ | — | 11 | 12 | 5 | 16 | 8 | 1 | 17 | 1 | 2+ | — | — | — | — | 6 | 6 | 5 | 7 | 5 | — | 12 | — |
| 11 | 31 | 34 | 34 | 56 | 35 | 14 | 18 | 36 | 37 | 35 | 32 | 35 | 46 | 12 | 16 | 5 | 5 | 0 | 71 | 3 | 16 | 16 | 35 | 23 | 2 | 7+7+ | — | 11 | 14 | 3 | 17 | 10 | — | 18 | 1 | 2+ | — | 4 | 35 | — | 4 | 7 | 4 | 4 | 4 | — | 9 | — |
| 12 | 5 | 36 | 35 | 44 | 29 | 12 | 13 | 36 | 33 | 33 | 30 | 35 | 51 | 6 | 15 | — | — | 0 | 70 | — | 8 | — | 35 | 19 | 0 | 8+7+ | — | 10 | 13 | 3 | 17 | 11 | — | 17 | 1 | 2+ | — | 0 | 34 | — | — | — | — | — | — | 0 | 9 | 0 |
| 13 | 31 | 35 | 33 | 52 | 34 | 16 | 13 | 35 | 32 | 33 | 28 | 32 | 45 | 5 | 17 | 5 | 5 | 0 | 68 | 2 | 11 | 2 | 34 | 21 | 3 | 6+7+ | 7 | 11 | 14 | 10 | 14 | 10 | 3 | 17 | 1 | 2+ | 2 | 2 | 35 | 5 | 6 | 6 | 5 | 5 | 5 | 0 | 8 | 0 |
| 14 | 10 | 37 | 36 | 61 | 37 | 23 | 15 | 36 | 34 | 30 | 26 | 31 | 48 | 10 | 16 | 4 | 5 | 0 | 72 | 0 | 22 | 0 | 35 | 20 | 4 | 6+7+ | 7 | 11 | 16 | 4 | 19 | 10 | 2 | 17 | 1 | 2+ | 2 | 5 | 25 | 5 | 0 | 6 | 2 | 4 | 2 | 0 | 10 | 0 |
| 15 | 12 | 37 | 36 | 59 | 44 | 17 | 13 | 38 | 33 | 38 | 30 | 33 | 51 | 11 | 20 | 5 | 5 | 0 | 77 | 8 | 22 | 15 | 32 | 22 | 3 | 6+7+ | 8 | 9 | 14 | 2 | 19 | 11 | 1 | 17 | 1 | 2+ | 2 | 0 | 37 | 5 | 7 | 7 | 3 | 5 | 3 | 0 | 12 | 4 |
| Average. | 22 | 36 | 35 | 53 | 36 | 16 | 14 | 36 | 34 | 34 | 30 | 34 | 49 | 10 | 17 | 5 | 5 | 1 | 71 | — | — | — | 34 | 21 | 3 | 7+7+ | 7 | 10 | 14 | 4 | 17 | 9 | 1 | 16 | 1 | 2+ | 2 | 3 | 31 | 5 | 5 | 6 | 3 | 4 | 2 | 2 | 10 | 2 |
| Extremes. | 5— | 31— | 31— | 43— | 19— | — | — | 34— | 32— | 30— | 27— | 31— | 44— | 5— | 15— | 5 | 5 | 0— | 63— | 0— | 8— | 0— | 31— | 18— | 6— | 7— | 8— | 12— | 3— | 13— | 7— | 1— | 14— | 1 | — | — | 0— | 25— | 5 | 0— | 5— | 0— | 3— | 0— | 0— | 8— | 0— |
| | 32 | 38 | 38 | 62 | 64 | — | — | 40 | 37 | 38 | 31 | 38 | 59 | 13 | 20 | | | 5 | 79 | 8 | 22 | 16 | 37 | 23 | 10+7+ | 8 | 12 | 16 | 10 | 19 | 11 | 3 | 16 | | | | 5 | 37 | | 7 | 9 | 5 | 7 | 5 | 12 | 12 | 12 |
| 16 | 0 | 33 | 33 | 42 | 28 | — | — | 33 | 33 | 34 | 27 | 27 | 40 | — | 14 | 0 | 3 | 0 | 73 | 0 | 3 | 0 | 25 | 18 | 0 | 6+7+ | — | 9 | 14 | 0 | 16 | 6 | 0 | 12 | 1 | — | — | — | — | — | 0 | 10 | 0 | 5 | 0 | — | — | — |
| 17 | 0 | 33 | 33 | 42 | — | — | — | 34 | 36 | 27 | 26 | 27 | — | 4 | 14 | 0 | 6 | 0 | 64 | 0 | 16 | 0 | 31 | 15 | 0 | 6+7+ | 7 | 9 | 14 | 2 | 11 | 6 | 0 | 12 | 1 | — | — | 0 | 25 | — | 0 | 8 | 0 | 7 | 0 | 0 | 11 | 0 |

marked in the nasal, dorsal, anal, and mandibular lines. In these lines they often reach a length of 2 mm., and are three and four parted at their ends.

## III. Structure of the Phosphorescent Organs.

Organs from different parts of the body have a common general structure, differing only in minor details. It will, therefore, be sufficient to describe a typical organ in detail and later compare with it those organs which have a specialized form or structure.

The epidermis of Porichthys has no scales and is richly supplied with large, club-shaped mucous cells in all stages of development. The dermis is quite a thick layer of dense connective tissue bearing blood vessels, nerves, and pigment cells. The phosphorescent organs are imbedded in the deeper portion of this dermis.

Each organ consists of four parts (Pl. XXXVIII, Fig. 4, and Pl. XXXIX[a], Figs. 5 and 9), the lens, the gland, the reflector, and pigment.

### 1. *The Lens.*

In a typical organ, from the anal or ventral row, for example, the outer or more superficial portion of the organ consists of a group of cells, the lens (Pl. XXXIX[a], Fig. 5, *l*). The surface of the lens directed toward the exterior, the distal portion, is oval or spherical in outline, while the deeper or proximal portion is projected into a more or less pronounced subconical form. The cells of the lens are polygonal in form in the center of the structure, becoming flattened toward the surface. At the distal surface the cells are quite regular and form a pavement-like layer (Pl. XXXIX[a], Figs. 5 and 9), but in the deeper conical portion the cells are very irregular in form, often with processes which interlace in a confused mass at the extreme proximal part. The lens cells have a small oval nucleus which is very sharply defined in contrast with the modified cytoplasm. The cell body is very dense, homogeneous, and highly refractive. The coagulation of this dense substance by reagents often slightly

separates the cells, thus rendering their outlines in sections very distinct.

The lens has no capillaries distributed to its substance. In Golgi preparations nerve fibers were found distributed to the superficial part of the lens and terminating among its cells in small free varicose ends. The number was, however, not greater than that found in the surrounding connective tissue of the skin and seems, therefore, of no special significance (Pl. XXXIX[a], Fig. 8).

### 2. *The Gland.*

The gland forms a shallow cup surrounding the proximal two-thirds of the lens. It is composed of cells varying greatly in size and shape (Pl. XXXIX[a], Figs. 5–7). The gland cells are held in a mesh of connective tissue and capillaries and in part by processes from the cells of the base of the lens. They have their long diameters placed vertical to that portion of the surface of the lens along which they lie. These cells have large round nuclei which are often vacuolated. The cytoplasm of the cells is very granular and is stained with the greatest difficulty. In alcoholic material these gland cells present an appearance which indicates that their granular constituents are largely dissolved out. In fact it is impossible to gain a true idea of the character of these cells from such material, as I found after many trials. On the other hand, the cells are beautifully preserved in Flemming's fluid and present in such preparations the structure shown in Pl. XXXIX[a], Figs. 6 and 7.

The gland is richly supplied with blood vessels, which enter around the distal border and also by puncturing the reflector below. The capillaries form a network among the gland cells and are in sections generally filled with red and white corpuscles.

### 3. *The Reflector.*

The reflector forms one of the most striking structures of the organ. It also is a cup-shaped mass which encloses the gland and the proximal portion of the lens, extending up around

the latter for over two-thirds of its surface (Pl. XXXVIII, Fig. 4, and Pl. XXXIX[a], Fig. 5). The reflector is composed of connective tissue, the matrix of which is modified into peculiar fine strands or fibrils, called spicules. These spicules very strongly reflect light. This property is very manifest even in the thinnest of sections where the reflector is dark gray or brown by direct light but bright silvery by reflected light. The spicules form a dense mass of fibrils somewhat regularly parallel with the surface of the lens. Small oval nuclei are scattered throughout the reflector, also a certain number of ordinary connective-tissue fibrils are scattered among the spicules, especially toward the periphery of the cup. That these fibrils are not "calcareous spicules," as von Lendenfeld describes for phosphorescent organs of Scopelus and other deep-sea forms, is evident, since they are not altered by nitric acid, nor, in fact, by any of the numerous fixing reagents used in their preparation.

Small blood vessels are found in the reflector, but only as they pierce the structure to reach the gland within.

### 4. *The Pigment.*

The pigment mass is composed of the large, many-branched type of cells characteristic of the pigment cells of the skin of fishes and amphibia generally. The cells are located around the outer and deeper surface of the reflector and vary in number in different organs. They are sometimes so numerous as to form dense masses, and again, as in the ventral or anal rows, there may be only three or four such cells to the organ.

## IV. Nerve Supply of the Phosphorescent Organs.

A most diligent and persistent effort was made to demonstrate the presence of a special nerve supply to the phosphorescent organs. Numerous preparations of the skin containing the organs were prepared by the methods which give specific nerve staining. By the iron-haematoxylin method and by the gold-chloride method, no nerves could be distinguished in the organs. By the Golgi method beautiful preparations were

obtained showing the distribution of nerves to the skin and to the epidermis, but in only two sections were nerves shown to have direct relation to the phosphorescent organ. The better one of these sections (Pl. XXXIX[a] Fig. 8) showed nerves branching over the surface of the lens. Whole mounts of the skin were made by the methylen-blue method of Bethe. These showed the most detailed network of nerve fibers lying in the connective tissue of the skin. Preparations containing both phosphorescent organs and lateral-line sense organs showed branches of the lateral-line nerve coming off the main stem and ending in the sense organs with almost diagrammatic regularity. Nerve fibers or bundles of nerve fibers were found in the skin above or below the phosphorescent organs, but no nerve bundles penetrated the phosphorescent organs and ended there. Two organs out of a very large number prepared contained each a single nerve fiber which entered the organ and terminated there. In one or two others single fibers seemed to terminate in the organ, but the fact could not be determined with certainty.

The facts set forth above, based on very favorable preparations, justify the conclusion that *the phosphorescent organs of Porichthys possess no specific nerve supply.* The few individual nerve fibers demonstrated to enter the organ may be considered as branches from the general nerve supply of the skin.

## V. Orientation of the Organs with Reference to the Surface of the Body.

A line passing through the middle of the three parts of the phosphorescent organ may be taken as its axis, and the position of this axis with reference to the body surface as the direction or position of the organ. In the ventral rows of the body the organs are directed downward, that is, the axes of the organs are almost or quite vertical with the surface of the body of the fish. The organs on the sides of the body and the lower part of the head are directed downward and slightly outward, and the axis of each organ thus makes quite a wide angle with the vertical to the surface. In the pleural row this angle is from

30° to 40°, while in the lower series of the lateral row it is often 80° or more ; in fact the axis is sometimes quite tangent to the surface of the body at that point. In this class of organs a variation from the typical arrangement of the parts may be noted. In a transverse section of such an organ, say from the pleural row, the deeper part of the cup of the reflector extends inward and upward (Pl. XXXIX[a], Fig. 10) ; that is, toward the dorsal part of the fish. It forms a somewhat deeper pocket than is formed by organs on the ventral surface. In such specimens the pigment is amassed around the upper or dorsal portion of the reflector. In these organs on the side of the body the conical base of the lens is relatively longer and is always in the axial line of the organ. The cells of the gland in such organs are arranged radially around the conical part of the lens.

The organs on the dorsal surface of the body and head have their axes vertical to the surface of the body. All these organs are small and rudimentary and irregular in their development, as shown by the fact that the lens is small and irregular in form, that the gland and reflector have more ordinary connective tissue in their structure, and especially by the inconstancy in the presence of the dorsal organs in different individual fishes.

## VI. Development of the Phosphorescent Organs.

The phosphorescent organs arise quite late in the development of the embryo. In skins of embryos 8.5 to 8.9 mm. in length, and in a transverse serial section of an embryo not measured but probably of about the same length, I find the first or incipient stages in the development of the rows of phosphorescent organs on the ventral surface of the body. The organs appear first in the ventral, gastric, branchiostegal, and apparently also in the anal rows at the same time. In the anal row it is not easy to determine their first appearance, owing to the changes accompanying the development of the fin.

In embryos 8 mm. in length the sensory Anlage of the lateral-line system is complete and the sense organs are sufficiently

well differentiated to be counted in skins. But the ventral and branchiostegal rows are not accompanied by sense organs, and, therefore, furnish a crucial test as to the origin of the organs. In the serial sections of the embryo mentioned above, the basal layer of cells of the epidermis of the ventral side of the body is slightly thickened in the region occupied by the adult ventral line (Pl. XL[a], Fig. 13). The thickening is produced by a more rapid increase in the number of the epidermal cells in the particular area.

In the gastric row of the same specimen there is a similar multiplication of cells just above the accompanying sensory Anlage (Pl. XL[a], Fig. 14). In this case the multiplication of cells shows a sort of center toward which the surrounding nuclei perceptibly converge. This is the beginning of a cell aggregation which soon proliferates into well-marked centers or groups, the antecedents of the individual organs of the line. In this incipient stage of the gastric organs the cells lie immediately against the cells of the sensory Anlage of the gastrogular row of sense organs, but they show no other evidence of origin from it. In skins it happened in one instance that the sensory Anlage of this row was torn free of the epidermis, and the cells along its upper border were apparently undisturbed. They were slightly increased in number, however, indicating a stage comparable to that from the ventral line figured in cross-section in Pl. XL[a], Fig. 13, or in the corresponding line in Pl. XL[a], Fig. 14.

Although it would seem impossible to affirm that the origin of the phosphorescent organs in the gastric row is independent of the sensory Anlage which has arisen by migration of cells, yet I am convinced that such is the case. The above facts, especially the independence of the origin of the rows above mentioned not associated with sense organs, form the basis of my belief that *the phosphorescent organs arise by local proliferation of cells from the epidermis in the region which they permanently occupy.*

The further progress in the development of the phosphorescent organ consists in the rapid multiplication of cells, giving rise to a distinct nodule which projects as a small papilla from

the inner surface of the epidermis (Pl. XL,[a] Figs. 15 and 16). The orientation of the organ is determined in this very early stage. Organs of the ventral line are vertical to the surface of the skin, while organs of the ventro-lateral surfaces are oblique (Pl. XL,[a] Figs. 15–20). In embryos 13 mm. long this papilla, in vertical section of ventral organs, presents the general outline of a finger-tip. It has a diameter of about six cells and is four to five cells deep. Pigment cells now appear in the connective tissue beneath the organ and are found in almost every section. In skins they show as the much-branched type of pigment cell spreading over the inner surface of the papilla. In specimens 14 mm. long there are from three to six such cells around each organ.

The next stage consists in the gradual separation of this papilla from the epidermis. The papilla becomes constricted where its sides are continuous with the general epidermis, the constriction continuing until complete separation occurs. At the same time a new layer of columnar cells forms in the epidermis and all evidence of the former union is obliterated. The separated mass now has a diameter of about .04 mm. and is found in embryos 18 to 20 mm. in length.

Soon after separation from the epidermis occurs, in fact before in some instances (Pl. XXXIX,[a] Fig. 12, and Pl. XL,[a] Fig. 21), the structure elongates slightly in the line of the axis of the developing organ and a differentiation occurs near the base, enabling one to distinguish in the mass two parts, an outer to become the lens and an inner the gland. No separation occurs between the two portions, yet the cells of each become more and more specialized in the direction of the cells of the adult lens and gland, respectively. By the time the embryo becomes free swimming, a length of about 25 mm., the organs possess the general characters of adult organs. Later growth consists in a great increase in size, due to the multiplication of the cells in the lens and gland, respectively.

Accompanying the differentiation of the lens and the gland there is a corresponding differentiation of the capsule. The connective-tissue cells of the dermis first form a cup-shaped aggregation around the base of the epidermal portion of the

developing organ (Pl. XL$^a$, Figs. 21, 23, and 24). The matrix of these connective-tissue cells is gradually converted into the modified fibrils which characterize the adult reflector (Pl. XXXIX$^a$, Figs. 9–11, and Pl. XL$^a$, Fig. 24). These fibrils are at first intermixed with a large amount of ordinary connective-tissue strands but ultimately form almost the entire mass. The pigment cells likewise increase in number and form masses of cells about the reflector, especially in organs on the side of the body.

Organs in different rows do not appear at the same time. Those on the mandible and ventral surface of the body appear first, and since they reach the highest development are taken as types. The lower series of the lateral line at 20 mm. is not farther advanced than organs of the ventral line at 11 to 12 mm. Those organs above the lateral line appear later and are always very rudimentary. In fact, such organs as I have designated rudimentary are never present above the lateral line and on the dorsal surface of the body up to the time when the embryos become free-swimming, a length of at least 25 mm.

## VII. Function of the Phosphorescent Organs.

I have kept specimens of Porichthys in aquaria at the Hopkins Seaside Laboratory, and have made numerous observations on them with an effort to secure ocular proof of the phosphorescence of the living active fish. The fish was observed in the dark when quiet and when violently excited, but, with a single exception, only negative results were obtained. Once a phosphorescent glow of scarcely perceptible intensity was observed when the fish was pressed against the side of the aquarium. Then, this is a shore fish and quite common, and one might suppose that so striking a phenomenon as it would present if these organs were phosphorescent in a small degree would be observed by ichthyologists in the field, or by fishermen, but diligent inquiry reveals no such evidence.

Notwithstanding the fact that Porichthys has been observed to voluntarily exhibit only the trace of phosphorescence mentioned above, still the organs which it possesses in such num-

bers are beyond doubt true phosphorescent organs, as the following observations will demonstrate.

A live fish put into an aquarium of seawater made alkaline with ammonia water, exhibited a most brilliant glow along the location of the well-developed organs. Not only did the lines of organs shine forth, but the individual organs themselves were distinguishable. The glow appeared after about five minutes, remained prominent for a few minutes, and then for twenty minutes gradually became weaker until it was scarcely perceptible. Rubbing the hand over the organs was followed always by a distinct increase in the phosphorescence. Pieces of the fish containing the organs taken five and six hours after the death of the animal became luminous upon treatment with ammonia water.

Electrical stimulation of the live fish was also tried with good success. The interrupted current from an induction coil was used, one electrode being fixed on the head over the brain or on the exposed spinal cord near the brain, and the other moved around on different parts of the body. No results followed relatively weak stimulation of the fish, although such currents produced violent contractions of the muscular system of the body. But when a current strong enough to be quite painful to the hands while handling the electrodes was used, then stimulation of the fish called forth a brilliant glow of light from apparently every well-developed organ in the body. All the lines on the ventral and lateral surfaces of the body glowed with a beautiful light, and continued to do so while the stimulation lasted. The single well-developed organ just back of and below the eye was especially prominent. No luminosity was observed in the region of the dorsal organs previously described as rudimentary in structure. I was also able to produce the same effect by galvanic stimulation, rapidly making and breaking the current by hand.

The light produced in Porichthys was, as near as could be determined by direct observation, a white light. When produced by electrical stimulation it did not suddenly reach its maximal intensity, but came in quite gradually and disappeared in the same way when the stimulation ceased. The light was

not a strong one, only strong enough to enable one to quite easily distinguish the apparatus used in the experiment.

An important fact brought out by the above experiment is that an electrical stimulation strong enough to most violently stimulate the nervous system, as shown by the violent contractions of the muscular system, may still be too weak to produce phosphorescence. This fact gives a physiological confirmation of the morphological result stated above that no specific nerves are distributed to the phosphorescent organs.

I can explain the action of the electrical current in these experiments only on the supposition that it produces its effect by direct action on the gland.

The experiments just related were all tried on specimens of the fish taken from under the rocks where they were guarding the young brood. Two specimens, however, taken by hooks from the deeper water of Monterey bay, could not be made to show phosphorescence either by electrical stimulation or by treatment with ammonia. These specimens did not have the high development of the system of mucous cells of the skin exhibited by the nesting fish. My observations were, however, not numerous enough to more than suggest the possibility of a seasonal high development of the phosphorescent organs.

Two of the most important parts of the organ have to do with the physical manipulation of light — the reflector and the lens, respectively. The property of the reflector needs no discussion other than to call attention to its enormous development. The lens cells are composed of a highly refractive substance, and the part as a whole gives every evidence of light refraction and condensation. The form of the lens gives a theoretical condensation of light at a very short focus. That such is in reality the case, I have proved conclusively by examination of fresh material. If the fresh fish be exposed to direct sunlight, there is a reflected spot of intense light from each phosphorescent organ. This spot is constant in position with reference to the sun in whatever position the fish be turned and is lost if the lens be dissected away and only the reflector left. With needles and a simple microscope it is comparatively

easy to free the lens from the surrounding tissue and to examine it directly. When thus freed and examined in normal saline, I have found by rough estimates that it condenses sunlight to a bright point a distance back of the lens of from one-fourth to one-half its diameter. I regret that I have been unable to make precise physical measurements.

The literature on the histological structure of known phosphorescent organs of fishes is rather meager and unsatisfactory. Von Lendenfeld describes twelve classes of phosphorescent organs from deep-sea fishes collected by the *Challenger* expedition. All of these, however, are greater or less modifications of one type. This type includes, according to von Lendenfeld's views, three essential parts, *i.e.*, a gland, phosphorescent cells, and a local ganglion. These parts may have added a reflector, a pigment layer, or both ; and all these may be simple or compounded in various ways, giving rise to the twelve classes. Blood vessels and nerves are distributed to the glandular portion. Of the twelve classes direct ocular proof is given for one, *i.e.*, ocellar organs of Scopelus which were observed by Willemoes Suhm at night to shine "like a star in the net." Von Lendenfeld says that the gland produces a secretion, and he supposes the light or phosphorescence to be produced either by the "burning or consuming" of this secretion by the phosphorescent cells, or else by some substance produced by the phosphorescent cells. Furthermore, he says that the phosphorescent cells act at the "will of the fish" and are excited to action by the local ganglion.

Some of these statements and conclusions seem insufficiently grounded, as, for example, the supposed action of the phosphorescent cells, and especially the control of the ganglion over them. In the first place, the relation between the ganglion and the central nervous system in the forms described by von Lendenfeld is very obscure, and the structure described as a ganglion, to judge from the figures and the text descriptions, may be wrongly identified. At least it is scarcely safe to ascribe ganglionic function to a group of adult cells so poorly preserved that only nuclei are to be distinguished. In the second place, no structural character is shown to belong to the

"phosphorescent cells" by which they may take part in the process ascribed to them.[1]

The action of the organs described by him may be explained on other grounds, and entirely independent of the so-called "ganglion cells" and of the "phosphorescent cells."

Phosphorescence as applied to the production of light by a living animal is, according to our present physiological-chemical notions, a chemical action, *an oxidation process*. The necessary conditions for producing it are two — an oxidizable substance that is luminous on oxidation, *i.e.*, a photogenic substance on the one hand, and the presence of free oxygen on the other. Every phosphorescent organ must have a mechanism for producing these two conditions; all other factors are only secondary and accessory. If the gland of a firefly can produce a substance that is oxidizable and luminous on oxidation, as shown as far back as 1828 by Faraday, and confirmed and extended recently by Watasé, it is conceivable, indeed probable, that phosphorescence in Scopelus and other deep-sea forms is produced in the same direct way, that is, by direct oxidation of the secretion of the gland found in each of at least ten of the twelve groups of organs described by von Lendenfeld. Free oxygen may be supplied directly from the blood in the capillaries distributed to the gland which he describes. The possibility of the regulation of the supply of blood carrying oxygen is analogous to what takes place in the firefly and is wholly adequate to account for any "flashes of light" "at the will of the fish."

In the phosphorescent organs of Porichthys, the only part the function of which cannot be explained on physical grounds is the group of cells called the gland. If the large granular cells of this portion of the structure (Pl. XXXVIII, Fig. 4, and Pl. XXXIX[a] Figs. 5–7) produce a secretion, as seems probable from the character of the cells and their behavior toward reagents, and this substance be oxidizable and luminous

[1] The cells which von Lendenfeld designates "phosphorescent cells", have as their peculiar characteristic a large, oval, highly refracting body imbedded in the protoplasm of the larger end of the clavate cells. These cells have nothing in common with the structure of the cells of the firefly known to be phosphorescent in nature. In fact, the true phosphorescent cells are more probably the "gland cells" found in ten of the twelve classes of organs which he describes.

in the presence of free oxygen, *i.e.*, photogenic, then we have the conditions necessary for a light-producing organ. The numerous capillaries distributed to the gland will supply free oxygen sufficient to meet the needs of the case. Light produced in the gland is ultimately all projected to the exterior, either directly from the luminous points in the gland or reflected outward by the reflector, the lens condensing all the rays into a definite pencil or slightly diverging cone. This explanation of the light-producing process rests on the assumption of a secretion product with certain specific characters. But comparing the organ with structures known to produce such a substance, *i.e.*, the glands of the firefly or the photospheres of Euphausia, it seems to me the assumption is not less certain than the assumption that twelve structures resembling each other in certain particulars have a common function to that proved for one only of the twelve.

I am inclined to the belief that whatever regulation of the action of the phosphorescent organ occurs is controlled by the regulation of the supply of free oxygen by the blood stream flowing through the organ; but, however this may be, the essential fact remains that the organs in Porichthys are true phosphorescent organs.

STANFORD UNIVERSITY, CAL.,
August 13, 1898.

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## EXPLANATION OF PLATE XXXVIII.

FIGS. 1 and 2. Lateral and ventral view of the lines of phosphorescent organs and lateral-line organs in the adult of Porichthys notatus. The representation of organs is diagrammatic. Small circles show the location of phosphorescent organs, and the dots the lateral-line organs. I am indebted to my friend, Edward Hughes, for the outlines upon which these two figures are constructed.

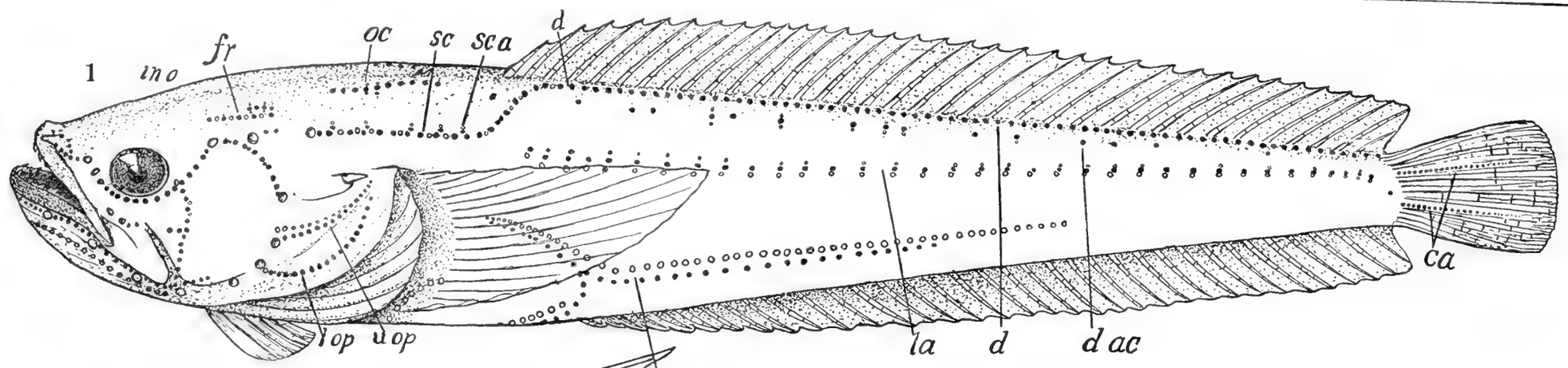
| | |
|---|---|
| *a* | anal. |
| *br* | branchiostegal. |
| *ca* | caudal. |
| *d* | dorsal. |
| *d ac* | dorsal accessory. |
| *fr* | frontal. |
| *ga* | gastric. |
| *ga gu* | gastro-gular. |
| *gu* | gular. |
| *la* | lateral. |
| *l op* | lower opercular. |
| *ma* | malar. |
| *md* | mandibular. |
| *mx* | maxillary. |
| *oc* | occipital. |
| *op m* | operculo-mandibular. |
| *pl* | pleural. |
| *sc* | scapular. |
| *sc ac* | scapular accessory. |
| *sup o* | supraorbital. |
| *t* | temporal. |
| *u op* | upper opercular. |
| *v* | ventral. |

FIG. 3. Lines and canals on the head. For explanation, see Figs. 1 and 2.

Figs. 4 to 24 are drawn with camera lucida. Figs. 4 to 8 represent the structure of adult phosphorescent organs, and Figs. 9 to 24 show the stages in the development of the phosphorescent organs of Porichthys notatus. The orientation of each organ with reference to the dorso-ventral plane of the fish is indicated by a short arrow pointing ventrally. Every section of epidermis shows numerous large mucous cells, many of which are empty.

FIG. 4. Cross-section of an adult organ in a new Porichthys from Panama. The gland shows several capillaries, all in cross-section but one. *l*, lens; *gl*, gland; *r*, reflector; *p*, pigment; *bl*, blood vessel.

1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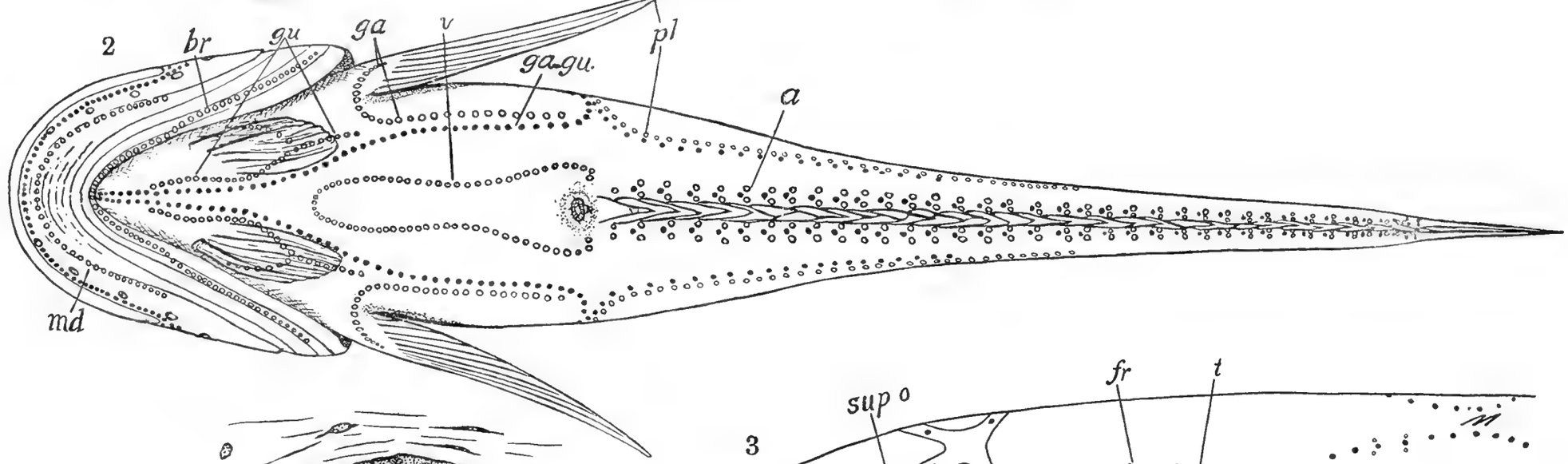
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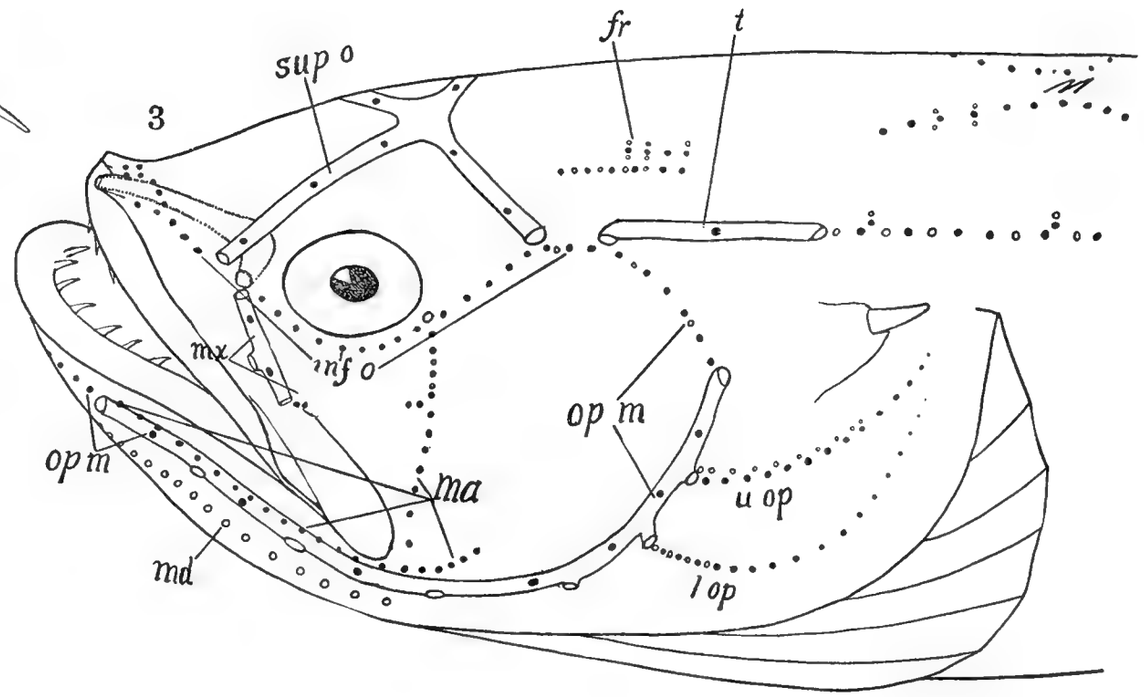
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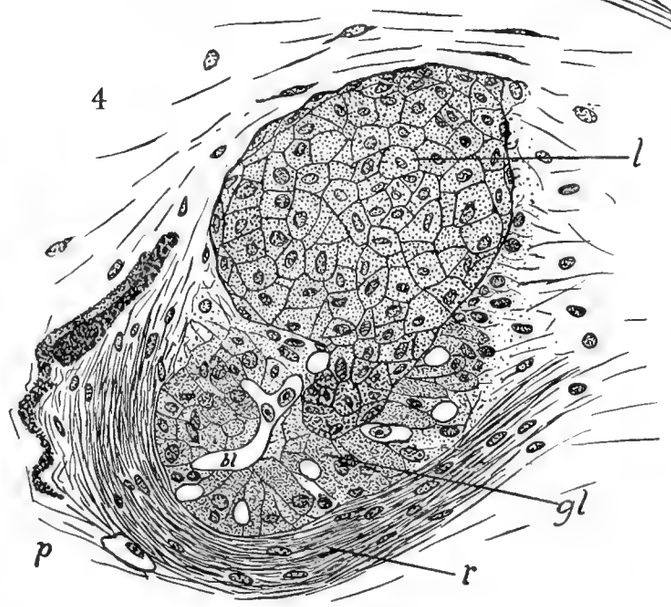
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## EXPLANATION OF PLATE XXXIX.[a]

FIG. 5. Cross-section of a ventral organ in Porichthys notatus Girard. *l*, lens; *gl*, gland; *r*, reflector; *bl*, blood vessels. In this section only a small amount of pigment shows to the left.

FIG. 6. Section of the deeper portion of a phosphorescent gland highly magnified to show the character of the cells. Flemming preparation.

FIG. 7. A somewhat oblique section of the deeper portion of a gland a little to one side of the center of an organ. Flemming preparation.

FIG. 8. The nerves distributed around the lens. Lens in outline. Golgi preparation.

FIGS. 9–11. Sections through a ventral, a pleural, and a lateral organ, respectively, of an embryo just before becoming free-swimming. The orientation of the organ with reference to the epidermis varies according to the position on the body. These three figures should follow in stage of development that shown in Fig. 24.

FIG. 12. Section of an embryonic organ just separating from the epidermis. This organ shows a differentiation even before it is cut off from the epidermis. Compare with Figs. 21 to 23.

5

l

gl

r

bl

6

bl

11

10

7

8

9

12

## EXPLANATION OF PLATE XL[a]

FIG. 13. Cross-section of a ventral organ at its first recognizable stage. Only a slight aggregation of cells in the basal layer of the ectoderm has appeared. This row is not associated with a lateral-line sense-organ Anlage. Embryo about 9 mm. long.

FIG. 14. Cross-section of gastric organ associated with sense-organ Anlage, *s an*. From the same embryo as Fig. 13.

FIG. 15. Cross-section of a pleural organ of an embryo 14 mm. long. The sensory Anlage shows to the left of organ.

FIG. 16. Section of a gular organ just external to the base of the ventral fin. Same embryo as Fig. 15.

FIG. 17. An organ from the same region as Fig. 16, but associated with a sense-organ.

FIG. 18. A pleural organ slightly older but from the same embryo as Fig. 15. *s an*, sensory Anlage.

FIG. 19. An inner anal organ beginning to separate from the epidermis. Same embryo as Fig. 15.

FIG. 20. Section through a pair of anal organs, showing a different degree of development. Same embryo as Fig. 15.

FIGS. 21 and 22. Organs in process of separation from the epidermis. Compare with Fig. 12.

FIGS. 23 and 24. Organs separated from the epidermis. The lens, *l*, and the gland, *gl*, are distinguishable, and the reflector, *r*, is forming from the connective tissue. Pigment cells, *p*, are arranged around the outside of the reflector.

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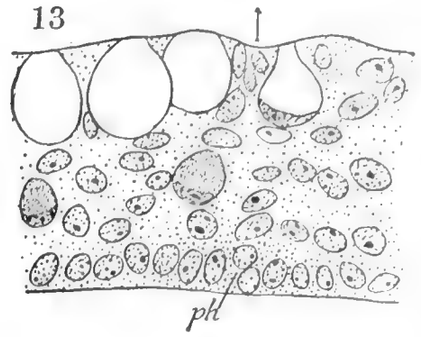


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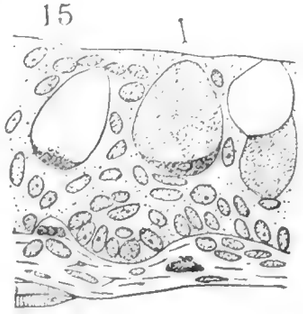

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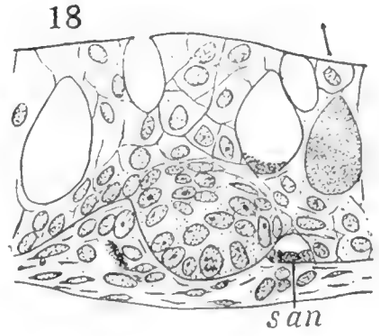


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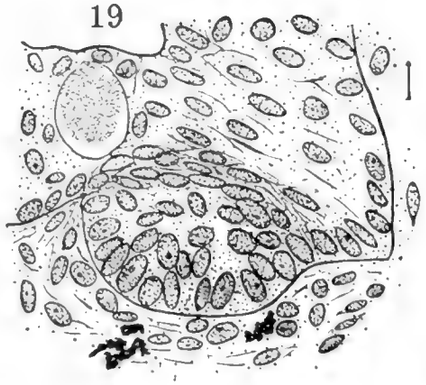

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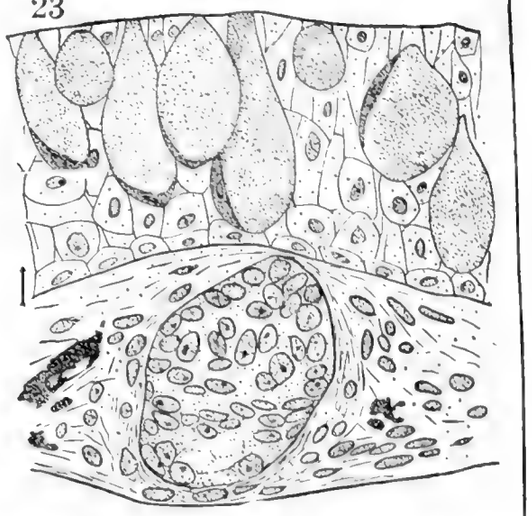

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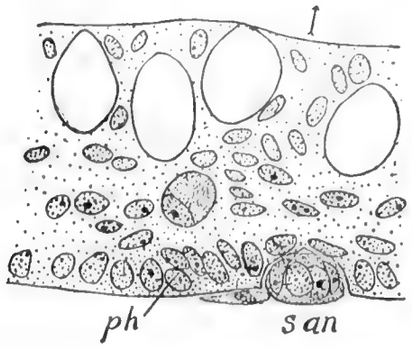


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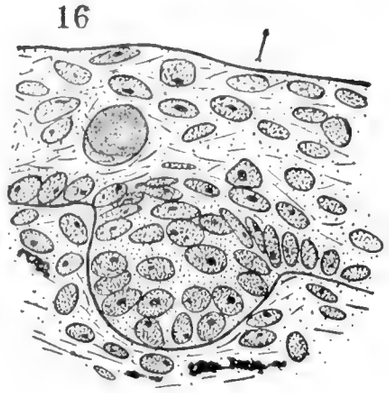

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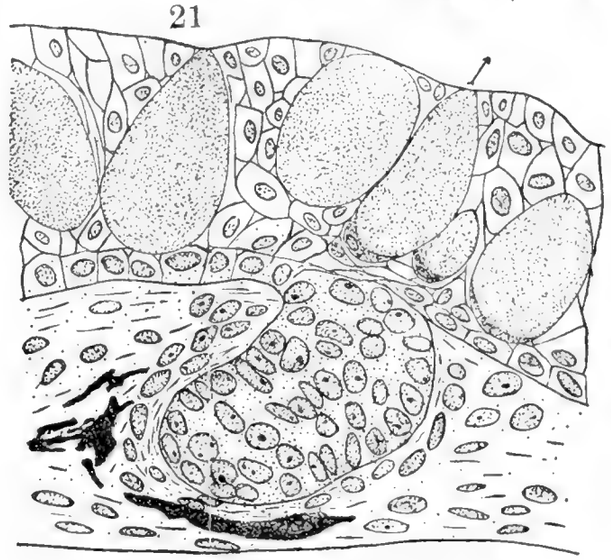

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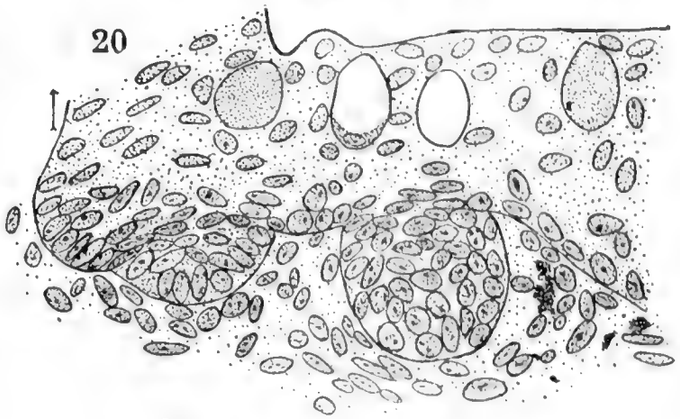

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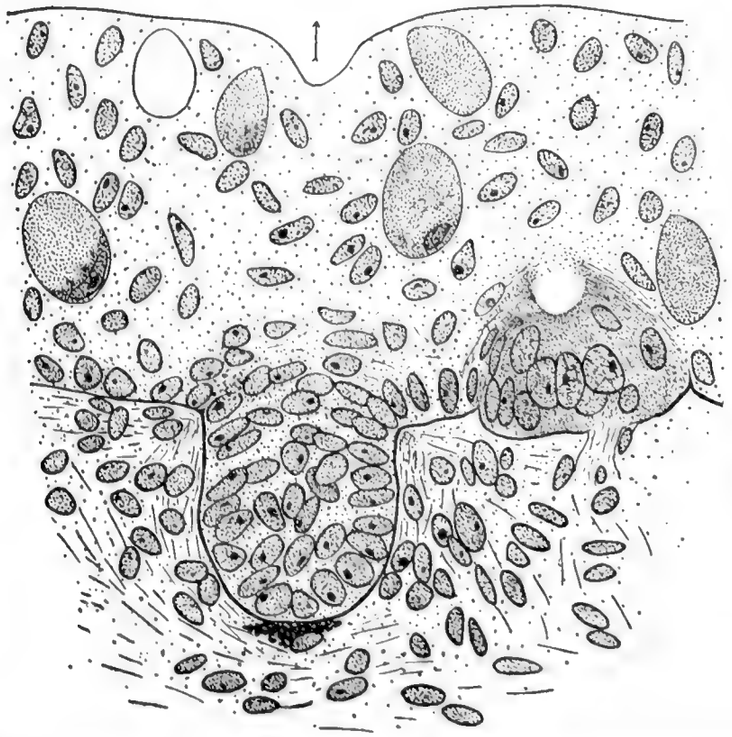

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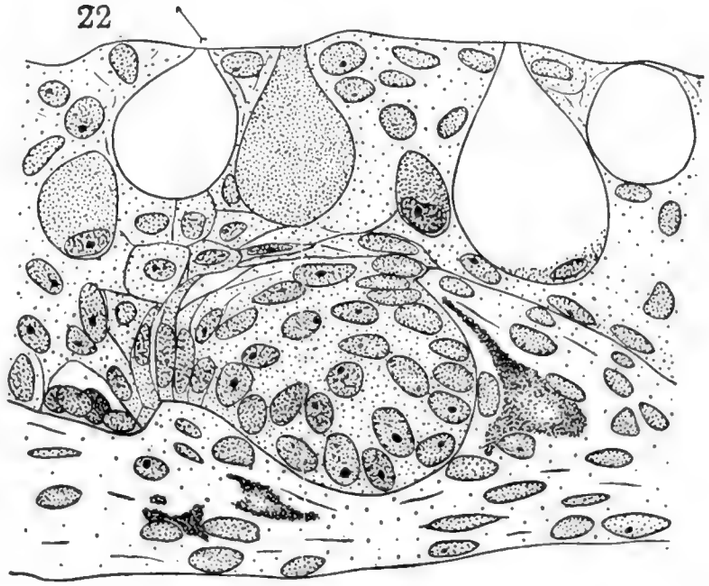

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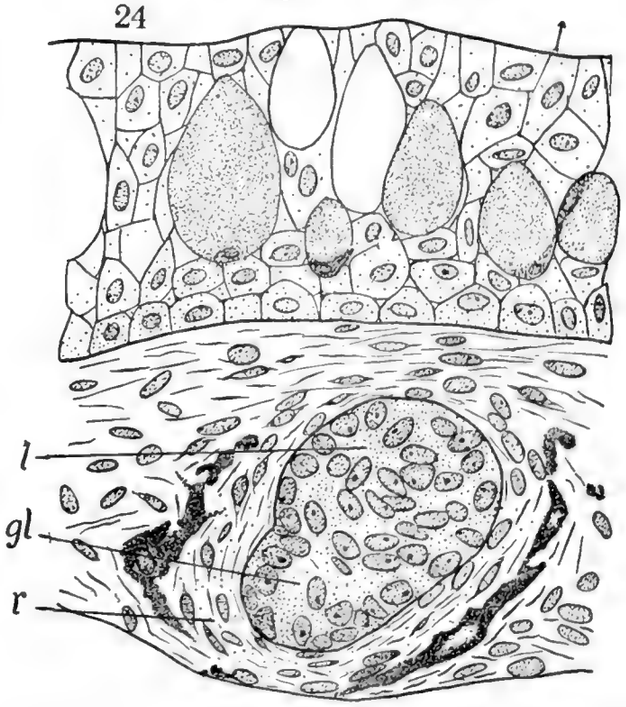

# ON THE SPECIES CLINOSTOMUM HETEROSTOMUM.

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Rudolphi (1) in 1809 described a worm found by one Andreas Jurine in the oesophagus of *Ardea purpurea*, and named it *Distomum heterostomum*. The main points in his description were briefly as follows: the worm measured 3 lines in length by 1 in width; the anterior fourth of its body formed a distinct neck; the anterior sucker, which was large, with a triangular aperture, was ventrally situated and subterminal — a swollen margin surrounded it. The ventral sucker, situated near the anterior, was smaller and deeper, with a more longitudinal aperture tending to be triangular. A third orifice, situated about one line behind this, was thought to give exit to the hidden cirrus. The body appeared transversely striated in preserved specimens: cirrus situated behind the ventral sucker.

In 1819 (2) he outlined this description in his *Synopsis*.

In 1845 Dujardin (3) gave a description evidently based on this one of Rudolphi, for he added no single point in the anatomy, and probably merely translated Rudolphi's words.

Diesing (4) in 1850 very briefly repeated this description without any further observations. He, however, gave another reference as to its habitat, — Rosa (5), — where it is said to be found under the tongue of *Ardea purpurea* as well as in the oesophagus.

R. Ramsay Wright (6) in 1879 described specimens from the mouth of *Botaurus minor* and gave a figure. He discussed the close relationship of *D. heterostomum* with *D. complanatum*, *D. hians*, and *D. dimorphum*, and suggested that the relative size of the suckers, on which much stress was laid in the differentiation of these species, may have been inaccurately observed, the prominent border about the mouth being taken for the anterior sucker; and in this suggestion one must agree with him entirely. He

corrected the old statement of the relation in size of the suckers in this form, giving the measurement of the anterior at 0.3 mm., and of the ventral at 0.8 mm. The pigmentation of the intestinal coeca and the existence of diverticula were described and a figure given. The main canals and caudal pore, as well as the subcuticular network of the excretory system, were described and the communication with the interstices of the parenchyma emphasized. The genital apparatus was partly worked out — vitellogens, testes, ovary, and a receptaculum seminis were mentioned, together with another structure situated anterior to the testes of unknown function. This was undoubtedly the cirrus. The measurements of the eggs were given at 0.099 mm. by 0.066 mm.

Von Linstow (7) in 1883 gave a brief description of the worm, making the measurement of the anterior sucker slightly larger than that of the ventral. He described the quadrangular sac filled with eggs which lies behind the acetabulum, and gave the measurements of the eggs at .11 mm. by .079 mm. He also mentioned the position of the two lobulated testes, the ovary and yolk-gland. His specimens were found in *Ardea nycticorax*.

Again (8), in 1886, in his description of some of the *Fedtschenko* material collected in Turkestan, he described and figured the worm. His description adds nothing to what was known of the anatomy, however, and the figure shows only the suckers, eggs, testes, ovary, part of the intestinal coeca, and a suggestion of the subcuticular excretory canals.

Stossich (9) in 1892 gave von Linstow's description.

Since this, Parona, in 1896, has mentioned the worm, but otherwise it has not been noticed.

My own observations on the anatomy of this form are as follows: Ten specimens were found adhering to the floor of the mouth and sides of the tongue of a great blue heron (*Ardea herodias*) shot on the Grand River at Dunnville, Ontario, Canada. They measured 6–10 mm. in length and 1–2 mm. in breadth during life, although, of course, the measurements vary greatly with the degree of contraction. They are of a dull reddish color, the intestines showing through as brownish black lines, one on each side of the body, and the testes and uterus,

with its contained eggs, as whitish masses. The body is flattened and hollowed ventrally, the neck being more terete and narrower, but also capable of being flattened and hollowed ventrally.

The anterior sucker is not large, but appears large from being surrounded by a mobile lip made up of a protrusion of the body wall. It is situated ventrally, slightly behind the anterior extremity. The acetabulum, situated some distance behind this, at the junction of the first and second thirds of the body, is larger than the anterior sucker in my specimens. At about the middle of the portion of the body behind the ventral sucker, the genital openings are seen close together, that of the female apparatus being directly in front of the male opening. The skin is unarmed.

The anterior sucker is situated on a slight eminence, and is entirely surrounded by the lip, which seems to assist in the physiological action of the sucker. The cavity runs upward and backward, and narrows suddenly into the very short oesophagus, which is provided with a muscular pharynx. The musculature of the sucker is composed of three layers, meridional, radial, and equatorial, or arcuate. The pharynx, similarly constructed and provided with a thick cuticular lining, opens directly dorsally into the much pigmented intestine, which branches almost immediately, sending the two coeca to the posterior end of the body. These coeca remain simple, but become much sacculated by the development of folds in the walls. The musculature of the intestine is limited to a single longitudinal layer. It is the arrangement of the mucosa with its extensive pigmentation, however, that offers one of the most striking characteristics of this species. The wall of the coecum is thrown up into high folds resembling somewhat the valvulae conniventes of the human intestine, and giving on section, when the coecum is cut tangentially, a ladder-like appearance. The epithelium is in a single layer, and consists of very tall cells tapering somewhat toward the lumen of the tube. These stain deeply at the base, where the rounded nucleus is situated (Pl. XXXIX, Fig. 6), but not so well centrally — possibly this appearance is partly due to their being thinner

there. They are densely loaded toward the free margin with granules and larger masses of a brown pigment.

The ventral sucker is larger than the anterior, and is situated somewhat deeply in the body of the worm, the margins appearing rather depressed. The musculature is as usual made up of three sets of fibers — an internal running in a somewhat triangular course parallel to the inner surface of the sucker, and provided with a triradiate fibrous intersection (Pl. XXXIX, Fig. 1) — a set of radial fibers making up the bulk of the sucker, and an external layer disposed in fasciculi over the surface of the sucker. The radial fibers sometimes cross in their course from the outer to the inner wall. In the external layer there is a fibrous point near the center, from which the fasciculi radiate backward; in front of this, however, they arch irregularly in a transverse direction.

There are no peculiarities in the arrangement of the body musculature; longitudinal, transverse, and two diagonal layers are present in their usual relations. There is, however, a reënforcement of the transverse layer in the neck region. The skin is quite unarmed and smooth, and I have been able to observe none of the transverse striations spoken of by some authors (Rudolphi (1), etc.). It is probable that this appearance was given by the delicate canals of the watervascular system, which form a rich network in the subcuticular tissues. The gland-like cells which lie under the superficial musculature seem more than usually numerous in this species, and form a thick layer. The parenchyma in the anterior portions of the body is made up of large oval cells with large round nucleus provided with a deeply staining nucleolus (Pl. XXXIX, Fig. 5). The protoplasm of these cells is very granular, the granules taking on a sharp eosin stain. In the posterior parts of the body the structure of the parenchyma is less apparent, and the definite cell bodies give place to a vacuolated reticular formation, with smaller scattered deeply staining nuclei.

The watervascular system is made up in this form of four parts — the ciliated funnels giving rise to fine tubules, the ciliated canals forming the subcuticular network, the lateral canals, also ciliated, and the paired caudal reservoir with the

caudal pore. The subcuticular canals joining one another for the most part at right angles, the transverse canals being much wider than the longitudinal, form a most conspicuous network, which may be injected with carmine by certain methods (R. R. Wright (6) ). A connection exists between this network and the lateral longitudinal canals which run at the sides external to the intestinal coeca. These lateral canals arise from two branches on each side anteriorly, the smaller joining the larger at about halfway, and posteriorly they run into pear-shaped dilatations which open by a common sphinctered terminal orifice. These dilatations near the opening are lined by rather high club-shaped epithelial cells.

The nervous system (Pl. XXXIX, Fig. 4) consists of a large commissure running dorsal to the muscular pharynx, and connecting two ganglionic swellings. From this are given off several small nerves to the anterior extremity, and backward there run four main trunks, two dorsal arising from the commissure, and two lateral running from the ganglionic swellings ventral to the coeca toward the posterior end of the body. A short distance behind the bifurcation of the intestine these ventral cords give off a branch which runs marginally backward. The finer ramifications of these nerves have not been traced.

We have left for consideration the genital apparatus. The male portion of this apparatus consists of two median testes with their vasa efferentia and a complicated cirrus.

The testes are large, and situated one behind the other in the median line, the left being anterior; their outline is not simple, but they tend to be lobulated. The vasa efferentia are wide and dilated at points with spermatozoa; they unite immediately before joining the cirrus sac. The penis (Pl. XXXIX, Fig. 3) consists of a long convoluted, rather thick-walled sac filled with spermatozoa, into which the vasa efferentia, or rather the short vas deferens formed by the union of these tubes, opens. This sac runs directly into a narrow, very muscular tube, which shows active peristaltic movements during life. At the end of this there is, apparently, a differentiated portion of the musculature which acts as a sphincter, making a dividing line between

this part of the tube and the dilated portion which follows, and which has a peculiarly modified cuticular lining. This dilated portion of the tube is surrounded by a thick layer of deeply staining cells very closely resembling those found in the general subcuticular tissues. The cuticular lining is furnished with large, highly refractive polygonal masses of cuticular substance. In sections these are sometimes detached. The subcuticular circular musculature is disposed much as it is in the general body surface. Narrowing again, this dilated portion passes to the external orifice, which is a crescentic slit immediately behind the opening of the female apparatus.

In the testes there is a somewhat alveolar structure, and the formation of spermatozoa seems to take place by the production of a morula-like mass from the dividing cell, the division products of the nucleus being peripheral; by the disintegration of this mass, as each nuclear particle is provided with a certain portion of the protoplasm, a number of spermatozoa are formed.

The female apparatus consists of an ovary, yolk-gland, Laurer's canal, and uterus, with their connecting ducts. The ovary is triangular and situated between the two testes on the right side. It is quite small, being less than one-fourth the size of either testis, and has a median position dorsoventrally in the body. It is somewhat lenticular in cross-section. The ciliated oviduct runs upward and forward from its anterior portion to join the other ducts leading to the uterus. The vitellarium, beginning at the level of the middle of the acetabulum, and extending back to the posterior end of the body, is made up of separate acini which send ductlets to form a transverse duct on each side, and these transverse ducts uniting in the center form a somewhat ventrally placed dilatation or reservoir. The nuclei of the cells making up the yolk-gland stain deeply with haematoxylin, while the yolk-granules stain brightly with eosin.

From the yolk-reservoir there are given off two tubes, one of which, running laterally, is thick-walled and widens into the uterus. The other tube runs forward and upward (this also is thick-walled and ciliated), and at a point where it becomes

dilated receives the oviduct, which has curved on itself and now runs backward and downward. The dilatation contains yolk-granules, spermatozoa, and occasional ova, and is continued into a third ciliated canal which runs forward and upward to open dorsally into the median line by a circular aperture. This is Laurer's canal.

The walls of the first dilatation of the uterine tube are very mobile, and may be considered to form an oötyp as the egg there receives the shell. It is surrounded by a number of large cells with large round nucleus and nucleolus, and deeply staining granular protoplasm which probably function as a shell gland, although such a relation could not be directly determined.

In many specimens the proximal portion of the uterine tube contains great numbers of spermatozoa which lie in the interstices between the eggs. The uterine tube is rather thin-walled and short, and after making a few convolutions in the area between the testes it runs forward to join at its left posterior angle the large quadrangular sac, which forms, when full of eggs, so conspicuous a feature in the living worm. The walls of the sac, which occupies the whole space between the acetabulum and the anterior testis, and the intestinal coeca laterally, are rather thicker than those of the uterus proper. They are like the rest of the uterine wall, underlaid by a layer of deeply staining cells, probably of a secretory nature. At its posterior end, directly in front of the anterior testis, this sac passes by a sphinctered opening into a muscular bulb-like vestibule, which opens again in the median line ventrally by the elongated sphinctered external orifice which lies directly in front of the male genital pore (Pl. XXXIX, Fig. 3).

The connections of these various ducts is made plain by the figure (Pl. XXXIX, Fig. 2) and need not be further described. I have not been able to make out any trace of a receptaculum seminis such as is described by Wright.

The eggs are large and numerous, and are provided with a thick shell and a well-marked operculum.

I have before me, through the kindness of Dr. Hassall, the type specimens of two forms which, as he has suggested, are

closely related to *D. heterostomum*. These were collected in various places, and from several different hosts. They are:

*Clinostomum gracile* Leidy.
*Distomum galactosomum* Leidy.

In addition to these, I have the specimens described by R. R. Wright, under the name *Distomum gracile*, from the branchiostegal membranes and fins of *Perca flavescens*. Another specimen, which I labeled *D. gracile*, I found encysted in the pectoral muscles of a frog in 1895. Other specimens found encysted in a trout have been determined by Stiles and Hassall as *Clinostomum heterostomum*. Finally, I have a number of specimens of an immature distome found encysted under the skin of *Ictalurus dugesi*, collected in Mexico. These, also, I have received from Dr. Hassall. All of these are immature forms.

In his original description of *Clinostomum gracile* (10), Leidy describes the prominent margin about the mouth-sucker, which is smaller than the acetabulum. The length is 3 lines, breadth 1 line. Habitat — intestine of *Esox*, and encysted in gills, fins, and muscles of *Pomotis vulgaris* and *Micropterus dolomieu*.

R. R. Wright (6) later identifies this form with the *D. gracile* of Diesing (12), which he describes from the perch. He gives a detailed description of the large acetabulum with its triangular aperture, but saw no trace of genital organs, and suggests that the sexually mature form might be found in some larger fish or piscivorous bird. Attention is called to the large pigmented intestinal coeca, and more particularly to the subcuticular meshwork of the watervascular system communicating with a wide median stem. Diesing's own description is not at all detailed.

Leidy's description of *D. galactosomum* (11) is briefly as follows: Habitat — *Roccus lineatus*. Anterior sucker surrounded by a prominent margin — body unarmed — ventral sucker sessile with triangular aperture; larger than anterior. Size 6–12 mm. by 2–2.5 mm. Intestines extend from the small pharynx to the tail, tortuous and sacculated. The animal has a reticular appearance, due to a network of opaque white lines communi-

cating with the lateral vessels running to a caudal vesicle; the white is due to granules of calcium carbonate, as it is removed by immersing in acetic acid. Generative apparatus is undeveloped.

On comparing the descriptions of these two forms, they are seen to differ in no respect whatever, unless it be in their hosts, which are still very similar.

Although I have not the type specimens for comparison, the description given by Looss (13) of *Distomum reticulatum* found encapsulated in the musculature of *Silurus glanis*, for which we have also the synonym *D. dictyotus* (Monticelli, '93), applies so exactly in every particular to the forms we have just considered, that I have not the least hesitation in concluding that they are the same. Indeed, this identity has been pointed out by Leuckart (14), who considers it one with Leidy's *Clinostomum gracile*.

The other specimens before me are identical forms, although none are definitely diagnosticated except those labeled by Stiles and Hassall, *Clinostomum heterostomum*. In short, there seems to me to be no doubt that all the specimens are individuals of one and the same form, and that that form is in reality a developmental stage of *D. heterostomum*.

My own observations on these specimens give the following additional points.

There is a slight variation in the stage of development of the different specimens, as may be judged from the differences in the maturity of the genital organs, but in all the two testes may be discerned with the ovary lying between them (Pl. XXXIX, Fig. 7), beside the coils of the very immature uterus. The lumen of the portion of the uterine tube running forward is quite narrow in proportion to the thick layer of deeply staining cells which surrounds it. Anteriorly it bends on itself, and enters the sac described in *D. heterostomum*, which is here a long, narrow, empty sac whose walls, furnished like the uterus with a thick layer of cellular tissue, seem relatively very thick. The cirrus is still very immature, although in one of my specimens (that from the pectoral muscle of the frog) the differentiation of its parts is fairly complete.

The intestinal coeca are not pigmented in any of these specimens, but the sacculated form is precisely that seen in the adult *D. heterostomum*.

In the specimens marked *Clinostomum heterostomum*, by Stiles and Hassall, the skin is provided with a very delicate armature of spines, but it seems to me, in view of the entire identity of structure in other respects, that this may be a variation due to the difference of hosts, or merely a temporary characteristic, and that this specimen is a different stage of the same form.

In every other respect these forms are entirely identical with the adult *D. heterostomum*, and it will be readily seen that the genital organs as described are precisely what would be expected in the developmental stages of this worm. It is on the ground of this identity in anatomical structure that I should class all these immature forms as developmental stages in the life history of *D. heterostomum*.

Several authors have suggested a close relationship between the adult *D. heterostomum* and the following other forms:

*D. hians* Rudolphi.
*D. complanatum* Rudolphi.
*D. dimorphum* Diesing.
*D. aquilae* Leidy.

Of these, *D. hians* and *D. complanatum* are readily distinguished by their having the genital pore in front of the acetabulum, and the ovary in front of the anterior testes.

*D. dimorphum* is described by Diesing (15) from two hosts, it being found in the muscle, body cavity, and intestine of certain fishes, and again, evidently in a more mature condition, in the mouth of *Ardea coçoi* and the oesophagus of *Ciconia americana*. The acetabulum is said to be smaller than the anterior sucker; the mouth terminal; genitalia are absent in the early stage. In the more adult the genital pore is not far from the caudal extremity. From this description, which seems to be the only one extant, and from his figures it is evident that this worm is closely related to *D. heterostomum;* indeed, it cannot be sharply distinguished, but as the original material is not accessible to me, I can go no further than this.

As to *D. aquilae*, one can judge but little from the description (11): "Spatulate, cochleariform, widest behind, obtuse at both ends; mouth circular, unarmed; acetabulum sessile about as large as the mouth. Length, 3 lines; width in front, $\frac{1}{2}$ a line; behind, $\frac{4}{5}$ a line. From the trachea of the Bald Eagle." The type specimen, however, shows a worm similar in every respect to that described as *D. heterostomum*, with the exception that the anterior sucker seems to be surrounded by no elevated border, and it is on this ground that I should hesitate to consider them identical without further examination of fresh material.

Up to this point I have employed the generic name, *Distomum*, in speaking of the form under consideration, by way of avoiding confusion, but according to the rules of nomenclature now generally adopted, this generic name can no longer stand, because (Railliet (16)) it is antedated by *Distomus* Gaertner, applied to a genus of tunicates, and further, as Stiles points out, because Retzius, in proposing it, arbitrarily created a synonym for the existing generic term *Fasciola*.

In the subdivisions generally adopted as a substitute for the genus *Distomum*, this form would fall into the section mesogonimus (Monticelli (17), '88), but Stiles and Hassall have pointed out that this, in turn, is antedated by the generic name *Clinostomum* (Leidy (10), '56), which was proposed to include a group of which the type species was *Clinostomum gracile*, a form identical with the *D. reticulatum* of Looss, on which Monticelli's genus was based.

Hence, in order to concur with the modern nomenclature, we can only name this form *Clinostomum heterostomum*, and it will now include the larval forms formerly known, respectively, as *Clinostomum gracile*, *D. galactosomum*, *D. reticulatum* Looss, and possibly also the adult *D. dimorphum* and *D. aquilae*.

In adopting this generic name, however, it is necessary that I should amplify the generic diagnosis given by Leidy (10) as follows (as suggested by Stiles):

Family, *Fasciolidae*.

Genus, *Clinostomum* Leidy ('56).

*Hermaphroditic flukes: Genital pore with contiguous male*

*and female openings situated posterior to the acetabulum, either about halfway between the acetabulum and posterior extremity* (*as in the type* Clinostomum gracile), *or immediately posterior to the acetabulum, as in* D. Westermanni, *Kerbert. Oral sucker without tentacles or spines: Caudal extremity without retractile appendage. Intestine simple, bifurcate.*

Thus there are contained in this description two groups of flukes, which will probably later be parted into separate genera.

I wish to express my thanks for literature, etc., to Prof. R. Ramsay Wright, of Toronto University, whose readiness to aid me in my work has been unfailing; also, to Drs. Stiles and Hassall, of Washington, whose library and specimens I have made use of, and who have been very kind in advising me with regard to the questions of nomenclature.

Baltimore, October 10, 1897.

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## DESCRIPTION OF PLATE XXXIX.

FIG. 1. Clinostomum heterostomum — adult seen from ventral surface; *ci.*, cirrus sac; *ov.*, ovary; *ex.*, excretory vesicle; *t.t.*, testes; *go.*, opening of uterine tract; *u.*, uterine sac; *vit.*, yolk gland.

FIG. 2. Female genital apparatus, seen from dorsal surface; *Lc.*, Laurer's canal; *od.*, oviduct; *yd.*, yolk ducts; *yr.*, yolk reservoir.

FIG. 3. Cirrus (from ventral surface); *v.e.*, vasa efferentia; *s*, sac containing spermatozoa; *c*, muscular tube; *p*, extrusible portion opening at external genital pore; *go.*, external female genital pore.

FIG. 4. Scheme of nervous system; nervous system in blue, alimentary tract in red.

FIG. 5. Parenchyma cells in anterior portion of the body, with strands of body musculature.

FIG. 6. Intestinal coeca in longitudinal section.

FIG. 7. Genital apparatus of immature form (D. gracile, etc.).


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# MITOSIS IN *NOCTILUCA MILIARIS* AND ITS BEARING ON THE NUCLEAR RELATIONS OF THE PROTOZOA AND METAZOA.

GARY N. CALKINS.

TABLE OF CONTENTS.



In view of the probable origin of the Metazoa from the colony-forming Protozoa, many observers have attempted, more or less definitely, to trace the phylogenetic development of special parts of metazoan cells from analogous parts in Protozoa. Prominent among such attempts have been those of Bütschli ('91), Heidenhain ('94), Hertwig ('96), and Lauterborn ('96), and the two last-named writers have shown that, from the least differentiated parts of the most primitive protozoan nuclei, a progressive series may be traced which culminates in *Nocti-*

*luca miliaris*, where the nucleus and the mitotic figures are quite as complex as in the Metazoa. Ishikawa ('94) had already shown the similarity between *Noctiluca* and the Metazoa in this respect, although his account of mitosis in the former was incomplete. *Noctiluca* holds so important a place in the series, especially in relation to the process of mitosis, that its nuclear division should be fully and accurately known, and it has been my aim during the last two years to clear up, if possible, the points still left obscure.

The wide distribution of *Noctiluca* and the large size of its nuclear elements have made it a favorite subject for research, although it has seldom been studied from a cytological standpoint. Huxley's description ('55) was purely morphological, as were those of De Quatrefages ('50), Krohn ('52), and Busch ('55). Brightwell ('57), describing the external phenomena of cell division, undoubtedly saw the sphere,[1] but called it a secondary nucleus. Cienkowsky ('71 and '73) described the external features of spore-formation, but without reference to the nucleus or the accompanying structures. Robin ('78) in his description of the spore-formation seems to have mistaken the sphere for a nucleus, and his accurate illustrations tell more about the nuclear processes of division than the text.

It was Ishikawa ('94) who first gave accurate details of mitosis in *Noctiluca*. His work can best be reviewed by presenting his own summary (pp. 324–326) as follows:

1. The division of the animal is preceded by the loss of the peristome, teeth, and the tentacle, the last of which is not thrown off, as Robin is inclined to think, but is redrawn into the body of the animal. The mouth and the "Staborgan" are, however, always present (Robin).

2. The spore-forming individuals differ from the dividing ones in not possessing the mouth and Staborgan in addition to the organs above mentioned, and by the excessively empty appearance of the cell interior (Cienkowsky).

3. The division of the nucleus is always preceded by a concentration of a part of the cytoplasm in the form of a spherical or oval granular body,

[1] I use the term "sphere" to designate the large, clearly outlined cytoplasmic mass which is probably to be identified as an attraction sphere. During the prophase of mitosis it divides and forms an amphiaster with connecting central-spindle fibers, and in the anaphase a centrosome is found within it.

mostly close to the nucleus. This is the archoplasm or kinetic center of division, and corresponds most probably to the "Nebenkern" of Von la Valette St. George.

4. In living animals at the stage of (3), the nucleus appears more or less homogeneous and transparent, and is not so distinctly to be seen as the archoplasm. But treated with reagents the chromosomes come into view distinctly.

5. Each chromosome consists of a row of disc-shaped microsomes irregularly scattered in the nucleoplasm. The number of the chromosomes is not clear, but in most cases has been counted to be ten.

6. The chromatin substance of each of the microsome-discs collects at the periphery and forms a microsome-ring.

7. In the nucleus of a dividing animal, each microsome-ring splits into half-rings, thus dividing a chromosome in halves, while in that of the spore-forming animals two successive divisions of a microsome-ring take place, so that a single chromosome is directly divided into four daughter ones.

8. The chromosomes collect on the side of the nucleus which is nearest to the archoplasm and spread out towards the other pole. The pole where the archoplasm lies thus corresponds to Rabl's "Polfeld," and the other pole to his "Gegenpol."

9. The archoplasm divides and forms a very large spindle which first lies tangential to the surface of the nucleus. This division of the archoplasm is succeeded by the separation of the chromosomes into two groups, each attracted (?) by its respective archoplasm.

10. The archoplasmic spindle thus formed pushes in the nuclear wall on which it lies, and the nucleus assumes in consequence a half-ring form.

11. By the separation of the archoplasms a spindle is produced which in all essential characters appears like the form known as the "dyaster stage," with a large archoplasmic mass at each end of the spindle.

12. The fibers of this spindle are therefore continuous from one pole to the other, and, lying outside the nuclear wall, become in no way connected with the chromosomes. But there is seen at this stage another set of fibers running from the center of the archoplasm to the polar ends of the chromosomes. This structure of the spindle corresponds exactly with that of the spermatocyte of *Salamandra maculata*, as investigated by Hermann, with only the difference of the persistence of the nuclear wall in *Noctiluca*, and the necessary modification in consequence of this fact. The optical appearance of these two kinds of fibers is different, just as in *Salamandra*.

13. Besides these two sets of fibers, the Verbindungsfaden are clearly to be recognized extending between the separating chromosomes.

14. The central-spindle fibers originate from the archoplasm, the radial fibers, probably from both the cyto- and nucleoplasms, and the Verbindungsfaden from the linin substance.

15. In the spore-buds the archoplasm is to be seen lying close to the nucleus up to the time of full development of the spore, just before its

detachment from the mother-animal, and a part of it becomes transformed into the flagellum, just as in many vegetable swarm-spores, as Strasburger shows.

16. In the center of the archoplasm is generally seen a centrosome, which often shows a dumb-bell form. Sometimes, however, two centrosomes are found in the archoplasm of the spore-forming cells. In many cases, again, there is found in the center of the archoplasm a number of small oval rod-shaped or curved bodies staining exactly like centrosomes, instead of one or two centrosomes. These may represent the group of centrosomes of Heidenhain.

17. The origin and the fate of the centrosome are not known. In a few instances it appears to be formed from the nucleus.[1]

Section 5 is rather obscurely worded, but, from the detailed description given elsewhere, it appears that the chromosomes, which are of various lengths, consist of irregular rows of microsomes lying scattered throughout the nucleus. "The chromosomes, except a few, do not seem to lie in definite order, but to be scattered more or less irregularly in the nucleus."

In the descriptive part of Ishikawa's paper section 7 is stated in a more conservative manner: "While the chromosomes of the nuclei of the dividing individual are represented by a double row of microsomes, those of the nuclei of the spore-forming individuals appear to consist of four rows." He explains this difference as follows: "In division the nucleus has to divide only once, and hence the chromosomes require only once to divide, while in the spore-formation divisions of the nucleus take place rapidly one after the other, and two divisions take place almost simultaneously" (*l. c.*, p. 304).

It may be pointed out, however, that in spore-forming individuals the nuclei continue to divide rapidly and without intervening resting periods, until there are from three to five hundred descendants of the original nucleus. What reason, therefore, can there be for quadruple division of the original chromosomes?

The details regarding formation of the nuclear plate and the separation of the chromosomes are not given.

[1] The killing agents used by Ishikawa were Flemming's stronger solution, picro-acetic, and acetic acids. The material was stained with Böhmer's haematoxylin, acid fuchsine, methylin blue, and methylin green.

Ishikawa speaks of "radial-fibers" (section 12), but as these are analogous in every way to similar fibers in ordinary mitosis, I see no reason for adopting this term in preference to the current name "mantle-fibers."

The present paper deals almost exclusively with the points touched upon by Ishikawa in sections 3–14, 16, and 17. The details of chromosome-formation are found to differ considerably from Ishikawa's account, and for the first time the formation of the nuclear plate in *Noctiluca* is described. Ishikawa's observations in regard to the sphere and centrosome are, in the main, confirmed.

Before giving the results of my observations I wish to express my obligation to Professor Wilson, not only for the material upon which most of my work was done, but also for his kind advice and unceasing interest.

## I. MATERIAL AND METHODS.

The material for my work was collected partly by Professor Wilson at Beaufort, N. C., in June, 1895, and partly by myself at Port Townsend, Puget Sound, in July, 1896. In the latter place it was found in great abundance, often covering hundreds of square yards, its presence indicated by brilliant phosphorescence by night, or in daylight, when wind and tide were favorable, by patches of brilliant orange.[1] They were collected early in the morning and preserved at intervals during the day and succeeding night, and all stages of vegetation, division, and spore-formation were thus secured. Five different killing agents were used, *viz.*, corrosive sublimate (saturated in normal salt solution), sublimate-acetic (10 per cent acetic), picro-acetic (Boveri's formula, with 1 per cent acetic for the Beaufort material, saturated picric with 5 per cent acetic for that from Puget Sound), Hermann's fluid, and Flemming's stronger solution.

The material was prepared for study either by mounting *in toto* or by imbedding in paraffin and sectioning. Considerable difficulty was experienced in handling such small objects until the following method was devised. The alcohol containing *Noctiluca* was poured into a glass cylinder two inches long and one-half an inch in diameter, covered at the lower end with bolting cloth of such fineness that the *Noctiluca* were held back while the alcohol passed through. They were then readily handled by car-

[1] *Noctiluca* was observed and collected in Alaskan waters as far north as Juneau and Sitka, and even in Glacier Bay, where the water is at a constant low temperature. The water of Puget Sound is deep and cold (about 51° at all depths), but at Sitka it is shallow and perceptibly warmer.

rying the tube from one staining pot to another. In all cases they were cleared in xylol and mounted in balsam. For sectioning, either large quantities of *Noctiluca* were imbedded in bulk, or individuals were selected and imbedded separately. In most cases the latter method gave the most satisfactory results, especially for dividing nuclei, where it was often desirable to section in definite planes. Heidenhain's iron haematoxylin, the Biondi-Ehrlich mixture, and Reinke's modification of the Flemming triple method gave the best results in staining. Bordeaux red, orange, and eosin were used as secondary stains with the iron haematoxylin.

## II. OBSERVATIONS.

### *A.* The Resting Nucleus.

The nuclei of *Noctiluca* belong to the so-called vesiculate type. They are spherical, oval, or elliptical in shape, and vary considerably in size, the largest measuring about 50$\mu$ in diameter (Fig. 6), the smallest about 30$\mu$, while those in the various stages of spore-formation are still smaller. They are always enclosed by a distinct, often thick, membrane, which, although it disappears at certain regions during mitosis, is, as a whole, retained through all phases of nuclear change.

The interior of the nucleus consists mainly of two distinct substances. One of these is granular and stains with acid dyes, while it is so abundant that it gives a massive appearance to the nucleus. The staining reactions of this substance show it to be the same, probably, as the "oxychromatin" described by Heidenhain ('94) in the nuclei of leucocytes. Unlike the latter, however, the oxychromatin granules in *Noctiluca* appear to be isolated bodies of large size and not suspended in a colorless network, the "linin" of Heidenhain. The other substance stains intensely green with the Biondi-Ehrlich mixture and represents the "chromatin" of Flemming ('80) or the "basichromatin" of Heidenhain. In the resting nucleus the basichromatin is invariably collected in from eight to eleven, or more, great chromatin reservoirs which, for the sake of brevity, may be called the karyosomes in place of the earlier and more misleading term, — nucleoli. By this use of the term, however, it must not be understood that the karyosomes are local thickenings of a general basichromatic reticulum. There is no such

network in *Noctiluca*, and "karyosomes" here represent the entire chromatic reticulum of metazoan cells. Karyosomes are characteristic of many Protozoa, and the nuclei containing them are classed together by Gruber ('84) as the "vesiculate" type ("Bläschenförmige"). In all such nuclei a membrane, nuclear ground substance, or "sap," and one or more central granules of chromatin can be distinguished.

In *Noctiluca* the two nuclear substances differ widely in their affinity for dyes, the oxychromatin being stained a clear and intense red by the Biondi-Ehrlich mixture, while the basichromatin is stained a brilliant green. This difference in color is so marked, and the limits of the two substances so distinct, that the smallest particles of green basichromatin can be easily detected on the red background of oxychromatin. This stain is, therefore, invaluable in following the chromatin changes during nuclear activity.

In addition to the basichromatin and oxychromatin, a small spherical body ($x$) surrounded by a hyaline area (Figs. 3, 4, 6, and 7) is usually to be found in the nucleus. With iron haematoxylin and orange it takes an even more brilliant haematoxylin stain than the chromatin, remaining black or blue while the karyosomes are a deep gray. I have not succeeded in staining it successfully with the Biondi-Ehrlich mixture. In most resting nuclei only one such body is present. From the subsequent changes in the nucleus, and from analogous bodies in other Protozoa, there is some reason to regard this corpuscle as the centrosome.

### *B.* The Sphere in the Resting Cell.

In the cytoplasm, outside of the resting nucleus, lies a large cytoplasmic mass which was first pictured by Allman ('72) and later described by Robin ('78) as a nucleus. From its relation to the nucleus and its behavior during cell division this cytoplasmic mass may be called the "attraction-sphere" or simply the "sphere," although in its resting stage no centrosome can be found in it. It is often as large as or larger than the nucleus (Figs. 1 and 8), but differs in appearance at different stages. It

is always in contact with the nucleus, sometimes having the form of a sphere, but more often that of an irregular mass with a darker peripheral and a lighter central portion. The latter appearance is caused by the accumulation of microsomes around the periphery, and by their absence in the central portion. In specimens fixed with Hermann's or Flemming's fluid the sphere is seen to have pseudopodia-like processes extending from it into the surrounding cytoplasm and passing imperceptibly into the reticulum (Fig. 9).

In addition to the microsomes in the sphere other deeply staining, but larger, granules are frequently seen. Sometimes only one of these can be found ; again they are quite numerous, even forming a small group of deeply staining bodies. Similar granules are also found in the cytoplasm, distributed throughout the reticulum. These granules are often strikingly similar to centrosomes and might easily be mistaken for them. Careful comparison of many spheres, however, both in sections and in total preparations, shows that they cannot be centrosomes. Their position is often eccentric (Fig. 5), sometimes even in the periphery of the sphere, while the presence of similar granules in the cytoplasm shows that they cannot be "centrosomes" in Heidenhain's sense. It is not improbable that the centrosomes described by Ishikawa, which were found sometimes single, sometimes double, and sometimes in groups, were cytoplasmic granules of this kind. Ishikawa apparently saw the "sphere" only during division : "The archoplasm, as we have stated, comes to be first seen at the stage a little before the division of the spore-formation" (*l. c.*). My observations, however, leave no doubt that it persists as an extra-nuclear mass throughout all periods.

### *C.* The Nucleus during Division.

The phenomena of nuclear division in *Noctiluca* are so complicated and apparently so different from typical mitosis in the Metazoa that the following general summary will help to make the details more clear.

During the early stages of nuclear activity the sphere divides into two similar halves connected by fibers which here, as in

some Metazoa, may be called the "central-spindle" (Figs. 9 and 11). The central-spindle then sinks into the nucleus which forms a trough to receive it (Fig. 13). The trough deepens and the lips of the nucleus approach each other over the central-spindle until the latter finally occupies the position *x* of the Fig. ⓧ, the daughter-spheres lying partly in the groove and partly exposed at the ends (Fig. 13). The nucleus then divides in a plane at right angles to the central-spindle, each half being accompanied by one of the daughter-spheres. The anaphase-stage presents the well-known striated appearance of protozoan mitotic figures — the striations being formed by the daughter-chromosomes which are directed towards the separating spheres (Fig. 18). In the final stage of division the daughter-nuclei become completely separated, the furrow in which the central-spindle had lain becomes obliterated, and the sphere resumes its normal appearance in the resting cell.

In spore-forming divisions the process is in the main the same, but the mitoses follow each other in quick succession and without intervening resting stages. Here the daughter-chromosomes of an anaphase without further change form the chromosomes of the ensuing prophase. The spheres again divide and the process is continued until the original nucleus is divided into as many parts as there will be spores (from 300 to 600).

### 1. *The Chromatin.*

*a. Prophase.* — Nuclear division is preceded by a concentration of the cytoplasmic microsomes in the sphere. The hyaline area disappears, the entire mass diminishes in size and becomes more homogeneous, and by these changes the sphere becomes more dense and more clearly defined, so as to be even more conspicuous than the nucleus. It was probably this stage which misled Brightwell ('57) and Robin ('78) into mistaking it for the nucleus.

Within the nucleus, meantime, the large basichromatic karyosomes gradually break up into smaller pieces, apparently in the same way as the chromatin in *Actinosphaerium* according to Gruber's ('83) and Brauer's ('94, '94a) descriptions. The disintegration of the karyosomes seems to be accomplished by a

progressive process of division, and from a study of the various modes of aggregation of the chromatin fragments in different nuclei, it appears that they divide first into two nearly equal parts, these into four, the four into eight, etc. In most cases the parts thus formed soon become scattered so that it is almost impossible to follow them ; but in some favorable cases the parts remain in groups in the places occupied by the original karyosomes until as late as the eight-part stage (Fig. 4). I have never been able to follow the fragmentation beyond this stage. The chromatin-granules thus formed leave their original positions and become concentrated at the side of the nucleus which lies away from the sphere (Fig. 5). But even here it can be made out that the larger pieces continue their fragmentation into smaller and smaller portions. The final result of this disintegration is the formation of a great number of minute chromatin granules similar to those of the nucleus of *Actinosphaerium* (Brauer).

As they break up, the chromatin masses often, but apparently not always, give rise to beaded fibers which have no definite arrangement in the nucleus (Figs. 6, 7, 12). Ishikawa ('94) in his second paper describes these fibers as chromosomes and pictures them in his Figs. 33, 38, 40, 44, and 47. He thought they were formed from the parts ("microsome-discs") of the disintegrated nucleoli (karyosomes) which were put together "one after the other like chains of mammalian blood corpuscles." I am convinced, however, that although these fibers appear in the manner described by Ishikawa, they are not the actual chromosomes. They do not pass directly into the nuclear plate, nor do they split longitudinally. This stage is a prophase of division, as shown by the condition of the sphere (Fig. 12). It is antecedent to the stage of the nuclear plate, and later than that of the resting nucleus. It must, therefore, correspond to the spireme-stage of mitosis, though it cannot be interpreted as a monospireme, for in total preparations the ends of the figures are in full view and are in no way connected with each other (Figs. 6, 7).

After the above changes the granules which compose the fibers of the spireme break down into still more minute

granules — the ultimate chromatin elements (Figs. 9, 10, and 36). These are so fine that one can distinguish them only after the most careful differential staining. At first they are distributed equally throughout the entire nucleus, but even before all of the chromatin of the karyosomes is converted into chromomeres, those which have already formed begin to collect in lines extending from the side of the nucleus which lies nearest the sphere, towards the opposite side (Figs. 9, 10, 32, 35, and 36). These lines of granules are the beginnings of the chromosomes. The Biondi-Ehrlich solution and the iron haematoxylin with orange G shows them best; the bright lines of chromatin granules show distinctly in contrast to the broad lines of oxychromatin lying between them. In sections stained by the first method (Fig. 10)[1] the minute green chromatin elements can be traced through all stages of chromosome-formation, from isolated granules of incredibly small size, distributed throughout the nucleus, to the compact chromosomes directed towards the sphere. The chromosomes next increase in thickness at the peripheral ends, probably by aggregation of the granules, and taper towards the center of the nucleus until at the inner end the threads are no thicker than the single granules (Fig. 11). The tapering of the chromosomes is, however, but a passing phase ; later they gradually thicken throughout their entire length and finally become of uniform thickness (Fig. 38).

Throughout all stages of nuclear activity there is no evidence to show that oxychromatin is being changed into basichromatin or *vice versa*. The chromatin granules are derived from the karyosomes, and at all stages can be distinguished from the oxychromatin granules by their intense green chromatin stain. The oxychromatin granules, meantime, do not change in size or in staining reaction. They are many times larger than the chromomeres, and during chromosome-formation they are arranged in lines parallel with the chromosomes (Figs. 9, 32, 36).

[1] These minute granules are difficult to represent in black and white and Fig. 10 represents but poorly the actual preparation. The intense black dots distributed throughout the nucleus and among the larger granules of oxychromatin give a fairly accurate picture.

When the latter are completely formed, these granules fill the remainder of the nucleus, the basichromatin of the chromosomes occupying no more space apparently than when in the form of karyosomes.

*b. Metaphase.*—At this period the chromosomes are single, thick rods or fibers of chromatin and distinctly granular, although each granule is much larger than one of the original elements. Shortly afterwards, however, the chromosomes become double, consisting then of two rows of granules formed by a longitudinal cleft down the middle line (Fig. 38). The main part of the chromosomes does not lie in the center of the nucleus as usual in other Protozoa (*Actinosphaerium*, *Euglypha*, etc.). On the contrary, the chromosomes lie in a linear group with their ends close against the membrane of the nucleus at the side nearest the sphere (Figs. 8, 11, 26). At this stage, or even before (Figs. 12 and 14), the nucleus begins to elongate in a direction which for convenience may be called the "primary axis," the ends of the chromosomes being arranged in the median line of the nucleus, that is, along the primary axis (Fig. 14). The nucleus thus assumes the appearance of a cylinder with rounded ends, the chromosomes extending from the margin nearest the sphere towards the opposite side (Fig. 14). This stage is rarely seen, however, for the elongate nucleus soon bends around the central-spindle to form a C-like figure (Figs. 12, 13). In many cases the curvature along the primary axis and the elongation of the nucleus are simultaneous and not separate actions. The curvature continues until the extremities of the primary axis become more or less closely approximated (Fig. 13).

By the curvature of the nucleus the chromosomes are carried around the central-spindle until they form an incomplete ring. They become focused at the point $x$ of the Fig. ⓒ, which is formed by the curvature of the nucleus. The axis in which the central-spindle lies may be called the "secondary axis" (Fig. 13).

*c. Anaphase.*—The structure of the chromosomes in the nuclear plate and their subsequent changes can be studied only in sections. These may be cut either transversely or longitu-

dinally, *i.e.*, in planes which pass through the secondary or the primary axis. In transverse section the chromosomes are thick, double fibers which tend to converge in the median line and to spread out from here like the ribs of a fan (Figs. 22, 26). At the proximal ends they are so compact that it is difficult to make out the double nature, but towards the middle the parts become more separated, and at the distal ends the granules in the chromosomes can hardly be distinguished from the remaining chromatin elements, which are still free in the nucleus.

Separation of the daughter-chromosomes begins at the proximal ends, *i.e.*, at the ends nearest the spindle. Each chromosome splits down the line of original longitudinal cleavage (Fig. 27, *A*), and the parts move in opposite directions, proximal ends first (Figs. 28, 29). Fig. 38 represents a section through the primary axis, showing the chromosomes in the nuclear plate in the metaphase. Fig. 27 shows the same stage in transverse section. In Fig. 27 the chromosomes have just begun to separate (*A*), while in Figs. 28, 29 the division is further advanced. During the separation which follows, some of the daughter-chromosomes, moving in opposite directions, pass each other, and a curious crossed appearance results (Figs. 28, 29). Fig. 30 is from a vertical section through the secondary axis, showing an anaphase; the chromosomes are turned in opposite directions towards the spheres. Fig. 31 represents the same stage in a section cut horizontally through the secondary axis.

As the daughter-chromosomes separate, the nucleus again elongates, but in a direction at right angles to that of its original elongation, *i.e.*, in the direction of the secondary axis (Figs. 5–20, 30). During this secondary elongation the nuclear lips are pressed more closely together above the central spindle until in some cases a mere slit is all that is left of the former opening (Fig. 17).

*d. Telophase.* — The description of nuclear division given above may apply equally to vegetative division or to that occurring in spore-formation. The telophase is, however, essentially different in the two cases. In the former, after the chromosomes are completely separated at the distal ends, the nucleus

itself begins to divide. At first this is indicated by a slight depression in the center of the nucleus (Fig. 20). The daughter-nuclei then move further apart until the connecting-piece is reduced to a mere thread, which soon breaks. The membrane reforms, and all traces of the groove are obliterated as each daughter-nucleus finally rounds out. The chromatin of the daughter-chromosomes fuses to form again the great karyosomes of the resting nucleus (Fig. 25), probably by a reversal of the process of disintegration described above. The distal elements of the daughter-chromosomes are the first to disappear, while the thicker parts remain as the last evidence of division (Fig. 25). In some instances the karyosomes begin to form before the daughter-nuclei are separated, as in Fig. 20.

In the spore-forming divisions, on the other hand, which follow each other in quick succession, the chromosome-structure is not altered, and the daughter-chromosomes pass directly into the nuclear plate of the next division figure. This process is repeated nine or ten times until all of the spores are formed. Fig. 21 represents the beginning of a second division before the first is quite complete, and with a relatively large "Verbindungsstück" left between the nuclei. The daughter-chromosomes which are to form the nuclear plate of the second division are single (Fig. 30), and yet just before the second division they are double (Fig. 38). This division must take place while the nuclear plate is forming. Ishikawa states that chromosomes are quadruple in nuclei destined to form spores, but I have never found the chromosomes in the nuclear plate otherwise than single or double. Even were they quadruple in the parent nucleus it would not suffice to explain the eight or nine subsequent divisions which the nuclei undergo before the spores are formed. The elements composing the chromosomes must divide longitudinally before each division of the nucleus. As there is no time for growth of the chromosomes between divisions, the quantity of chromatin in the nucleus is constantly reduced by half, and the nuclei become smaller and smaller.

### 2. *The Nuclear Membrane.*

In the resting cell the nuclear membrane is comparatively distinct and thick, but in the active nucleus it becomes very much thinner and more plastic. Except in one region it does not disappear during mitosis, but persists as a permanent portion of the nucleus. In one region, however, it does disappear during mitosis. When the nuclear plate is formed and the chromosomes are ready for division, it disappears in the region between the nuclear plate and the central-spindle. The nuclear plate is thus left as a free ring around the central spindle (Figs. 13, 29, 31). As the daughter-chromosomes separate, the membrane is reformed between them (Fig. 30), but it always remains broken in the region between the daughter-chromosomes and the spheres. The position of the gap which is thus formed in the membrane can be clearly seen from Fig. 31. Here the membrane is complete in all places except where the mantle-fibers pass from the centrosomes to the chromosomes. During the telophase the membrane reforms in the broken places and again becomes continuous.

The above-described gap in the membrane during mitosis is strikingly similar to a temporary stage in metazoan mitosis which has been described by many observers. In all such cases the nuclear membrane disappears first at the poles in front of the developing spindle-fibers. In some instances (*e.g.*, in *Thallassema* Griffin ('96) ) the membrane persists in this stage for some time, and only at a late period in mitosis does it completely disappear.

### 3. *The Sphere.*

We may now consider the history of the sphere more in detail. As previously stated, the approach of division is first recognized by the concentration of cytoplasmic microsomes in the sphere. During disintegration of the karyosomes and formation of the chromosomes a structure is formed consisting of two daughter-spheres connected by a "central-spindle," to which, from analogy with metazoan mitoses, the name of amphiaster may be given (Figs. 9, 11). As the nucleus elon-

gates and bends in the plane of the primary axis, the central-spindle sinks into the depression which is thus formed, until it finally occupies the position $x$ in the Fig. ℭ. The spindle thus lies in the secondary axis of the nucleus which encircles it, the spheres alone remaining outside (Figs. 13–15). The nuclear plate is wrapped around the spindle like a ring, the chromosomes lying midway between the two poles. The central-spindle fibers are at first not straight, but run from pole to pole in curved lines (Fig. 11). This curvature is caused by the spheres moving around the nucleus through an arc of 90°, the fibers becoming straight only when the central-spindle finally lies in its definitive position (Fig. 13). In some cases the bend of the central-spindle is much exaggerated until an acute angle is formed. During this time the growing chromosomes are entirely separated from the central-spindle, but when the nuclear plate is complete the intervening membrane disappears, and then, as in the metazoan spindle, the chromosomes lie directly upon the central-spindle. After the preliminary arrangement of the elements of the mitotic figure, the central-spindle undergoes a considerable elongation (Figs. 17–19, 30), while at the same time the nucleus is drawn out in the direction of its secondary axis in the form of a hollow cylinder with a ring of chromosomes at either end (Figs. 17–19). The dumb-bell-shaped amphiaster lies in the hollow with the spheres projecting at either end (Fig. 13). After division of the vegetative nucleus the sphere returns to its resting condition (Fig. 20). It becomes less dense and less homogeneous, and the central part more or less hyaline, while the peripheral portion still retains its granular aspect. The transformation may begin even before the nuclei are completely separated. Such a condition is indicated in Fig. 20, where the daughter-spheres, having lost their densely granular spherical condition, have become more diffuse, and have acquired the characteristic appearance of the resting spheres. On the other hand, after spore-forming divisions, the daughter-spheres do not return to the resting condition. They retain their spherical shape and dense granulation (Figs. 17–19), and soon divide again for the ensuing mitosis. It frequently happens that this secondary division of

the daughter-spheres takes place before the daughter-nuclei of the preceding mitosis are completely separated (Fig. 21). Fig. 21 also shows that the plane of the second division of each sphere is at right angles to the plane of the first division. The same thing is shown in an earlier stage in Fig. 18, where the primary axis of the daughter-nucleus is bending around the sphere; and again in the sections represented in Figs. 24, 39.

In some cases, particularly after fixation with Hermann's fluid, thick but short fibrous processes may be seen passing from the sphere into the cytoplasm (Figs. 9 and 11). These are analogous to the astral rays of metazoan cells, and like the central-spindle fibers they are formed from the substance of the sphere, being made up of granules or microsomes similar to those found in the sphere.

According to the foregoing description, the sphere in *Noctiluca* apparently corresponds to Boveri's archoplasm ('88). It is a persistent cytoplasmic structure, of a definite size and shape in resting and active cells, and appears to consist of a specific substance. Astral rays and central-spindle are formed from its substance, while at certain stages a centrosome lies within it (see p. 731).

The main observations by contemporary writers on the sphere fall into one or other of two groups, according as they agree with Van Beneden's or Boveri's conception of the origin of this structure. Van Beneden thinks that the sphere, while it consists of the same substance, is morphologically different from the cell plasm. Boveri originally considered it (and with it all spindle and astral fibers) as not only different from the cell plasm morphologically, but as composed of an independent substance — the archoplasm. Among botanists the "archoplasm idea" is generally accepted, and is expressed by Strasburger's term "kinoplasma," while among zoölogists this theory has not been generally approved, most observers supporting either Van Beneden's theory or some modification of it. There is, however, the greatest latitude in this support. Many interpret the sphere as formed anew from the cytoplasmic reticulum at each mitosis. (See among others Heidenhain ('94), Reinke ('94), Wilson ('95), Eismond ('95), Kostanecki

('96), v. Erlanger ('97), etc.) Others, however, while regarding the sphere as a differentiation of the cytoplasmic reticulum, find that it is permanent in the cell, dividing by fission like the centrosome. (Van Beneden '87, Meves '95, vom Rath '95.) Here the two views stand rather close together; on the one hand, it is maintained that there is a distinct structure permanent in the cell, but composed of modified cytoplasmic reticulum; on the other hand, it is held that the distinct structure is composed of a distinct substance. The difference here is certainly not great.[1]

The fact that in some cases the sphere appears only during mitosis is perhaps the most serious obstacle to Boveri's interpretation. He avoids it, however, by maintaining ('96) that the archoplasm is not necessarily restricted to a definite body, but may exist throughout the cell in the form of minute granules, which during mitosis are attracted around the centrosomes, forming spheres. He now ('97) considers that the eggs of *Ascaris*, upon which his idea was based, differ from other cells in regard to the presence and form of the archoplasm, the general rule being that fibers run directly from the centrosome into the cytoplasm. He also says that with the exception of *Noctiluca* (Ishikawa) he knows of no other form of cell where a solid archoplasmic substance exists around the centrosome.[2]

Schaudinn's *Paramoeba* must now be included with *Noctiluca*. Kostanecki's ('96) work on *Ascaris* does not sustain Boveri's conception, since he finds that, as in other forms, the spindle-fibers pass directly from the centrosome into the cytoplasm; and his work shows that in regard to archoplasm this form cannot be classed with *Noctiluca* and *Paramoeba*.

[1] In a number of cases the term "archoplasm" has been used to designate portions of the cell which are not obviously homologous with the parts described by Boveri. Thus Foot ('96) calls everything in the cell which stains with Lyons blue "archoplasm," and Moore ('94) apparently restricts the term to the residual spindle-fibers (Nebenkern).

[2] "Ja, mit Ausnahme von *Noctiluca*, wo das Archoplasma nach Ishikawa genau den Eindruck jener körnigen Substanz des *Ascaris*-Eies macht, wüsste ich keinen anderen Fall zu nennen, wo zunächst im Umkreis des Centrosoms eine dichte körnige Kugel besteht, die sich allmählich in das Strahlensystem umwandelt" (*l. c.*, p. 40).

It appears, therefore, that the archoplasm-theory in its original form is far from being definitely established. Heidenhain ('94, '97), one of the chief opponents of the archoplasm idea, has, however, described a substance connected with the centrosome which is of considerable interest in relation to that theory. In resting cells this substance is described as follows: "Diese Centralkörper sind innerhalb des Microcentrums durch eine Zwischenmasse miteinander verbunden, welche meist recht gut sichtbar ist, und im günstigen Fall durch das Rubin der Präparate stark gefärbt erscheint. Diese Zwischenmasse entspricht ganz genau der Materie der 'primären Centrodesmose' beim Leukocyten" ('97, p. 231). It is composed of minute granules, and from it are developed the fibers of the central-spindle which form the secondary "Centrodesmus." This substance would seem to agree to a certain extent with the "archoplasm" of Boveri's theory, and I believe that it may be compared to the substance of the sphere in *Noctiluca*. Like the centrodesmus, the sphere is made up of granules surrounding the centrosome, and during mitosis the central-spindle fibers are formed from it. Like archoplasm, it is a specific substance in the cell, surrounding the centrosome and giving rise to the spindle-fibers. While Boveri's original conception of archoplasm as a distinct substance in the cell may not hold for the Metazoa, it is, I believe, entirely consistent with the facts in Protozoa, as will be shown in the comparative part of this paper.[1]

### 4. *The Mantle-fibers.*

It has been shown above that the fibers of the central-spindle have no connection with the chromosomes, but pass without interruption from pole to pole. In the metaphase, and after the disappearance of the membrane, a second set of spindle-fibers is formed, passing from the sphere to the ends of the chromosomes. These are the mantle-fibers or "radial-fibers" of Ishikawa. He speaks of them as follows: "Of the origin of the mantle-fibers in *Noctiluca*, I can say but a few words,

[1] *Cf.* p. 755.

since the whole problem remains obscure and requires a thorough study with better methods and optical instruments. In sections given above, these fibers which are formed within the nucleus and probably attached to the chromosomes appear to come into close juxtaposition, but not to be continuous with those without, *i.e.*, those seen within and without the nucleus appear to be different from each other, the former originating from the nucleoplasm and the latter from the cytoplasm, just as Brauer thinks concerning the formation of the spindle-fibers of *Ascaris megalocephala bivalens*" (p. 323). My observations, while not conclusive, show, I believe, that the mantle-fibers (or "radial-fibers" of Ishikawa) are connected with the "nucleoplasm," but lie completely outside of the nucleus in the substance of the sphere. The mantle-fibers in mitotic figures of most Metazoa are usually first visible just before or during the time of disappearance of the nuclear membrane. So it is with *Noctiluca*, but here, as in the first stages of spindle-formation in various eggs, the membrane, instead of disappearing entirely, fades away in one part only.

Much has been written during the last few years on the origin of the spindle-fibers, the main question being whether they are of nuclear or cytoplasmic origin. In the different cells of Metazoa the fibers arise sometimes from the nucleus, sometimes from the cytoplasm. In other cases, *i.e.*, when the mitotic figure contains a central-spindle and mantle-fibers, they arise from both nucleus and cytoplasm, in which case the central-spindle usually comes from the cytoplasm (Hermann ('91), Meves ('96), Flemming ('95), Heidenhain ('94), Ishikawa ('94) ).[1] In many cases, however, no central-spindle can be distinguished, and in such forms the fibers sometimes arise in the cytoplasm (Strasburger ('92, '97), Mottier ('97), Osterhaut ('97), and botanists in general; Griffin ('96), Wheeler ('95), Mead ('97), etc.), or, in many cases, from the nucleus (Weismann and Ishikawa ('89), Brauer ('93, '94), Rückert ('94), Korschelt ('95), Wilson ('96), v. Erlanger ('97) ).

I have already shown that the fibers of the central-spindle

[1] An exception is found in *Ascaris megalocephala univalens* where the central-spindle is intra-nuclear (Brauer ('93) ).

in *Noctiluca* are formed from the substance of the sphere, or archoplasm. The mantle-fibers are probably nuclear in origin, as indicated by Ishikawa. They are focused in the centrosome and connect with the chromosomes. The microsomes composing them are at first only loosely strung together (Fig. 38), but later they become more closely packed, forming solid fibers (Figs. 30–39). Since the chromosomes lie in a ring around the central-spindle the mantle-fibers also surround it, and thus complete the similarity to some mitotic figures in Metazoa (Figs. 1, 28, and 29).

When fully formed, the mantle-fibers seem to be stiff filaments of constant length and thickness. They do not shorten as mitosis progresses, nor do they become thicker, but in the telophase they appear the same as in the earlier anaphase (Fig. 24).

### 5. *The Centrosome.*

It is with some hesitation that I undertake a description of the centrosomes in *Noctiluca*, for they are so minute and are accompanied by so many large cytoplasmic granules that, except in mitosis, their identification is not only difficult, but sometimes impossible. I am certain that Ishikawa, in many cases at least, mistook some of the cytoplasmic granules for centrosomes, for I have been entirely unable to find centrosomes of the size and form described by him. Nevertheless, his general conclusion that a centrosome in *Noctiluca* does exist is correct ; for its identification when connected with the mantle-fibers presents no difficulty whatever (Figs. 23, 24, 28, and 38). In regard to its origin Ishikawa had little to say, but he surmised that, in some cases at least, it comes from the nucleus.

While fully appreciating the difficulties in the way of determining the origin of a body so minute and so easily confounded with other granules, there are, nevertheless, several phenomena connected with the prophase of nuclear activity which give, I believe, an apparent basis for the conclusion that the centrosome is a minute granule lying inside the nucleus of the resting cell, and that it migrates out into the attraction-sphere during division.

In the metaphase the centrosome can easily be identified in all nuclei, whether undergoing vegetative or spore-forming division, and in the latter it can be distinguished at all stages. In spore-forming mitoses it divides early in the anaphase so that single centrosomes are rarely seen. I have not observed its division in the anaphase of vegetative mitoses ; it probably remains undivided until the prophase of the next division. In the metaphase the double centrosome lies in the inside of the sphere, but usually in an eccentric position (Fig. 38). In Fig. 38 (a mitosis in a spore-forming cell) the two daughter-centrosomes lie close together and separation would take place later in a direction at right angles to the plane of the paper. The preceding division is just finished, and the daughter-centrosomes have divided for the ensuing mitosis.

The daughter-centrosomes divide in the early anaphase, after which they lie more nearly in the center of the sphere (Figs. 28 and 31), and, as a rule, they remain near the center until the division is complete (Figs. 23 and 39). They may lie eccentricly, however, even in the telophase (Fig. 24), but in this case it is away from the nucleus. Except for the mantle-fibers, the centrosome is in no way the center of a radial system ; the sphere lies around it as an inert undifferentiated mass.

The centrosome appears to vary somewhat in form and in size. In some cases it is comparatively large and round (Fig. 24) ; in others it is round but much smaller (Figs. 23, 39); while in still other cases it is irregular in outline or even triangular (Fig. 38).

In the resting cytoplasm I have failed completely to find a centrosome in the sphere. In the resting nucleus, however, there is a minute body which stains intensely with the iron haematoxylin and which differs considerably in appearance from chromatin granules (see p. 7). This body disappears during the chromosome-formation and is not seen again until the nucleus reforms after division (compare Figs. 3, 4, 6, 7 with 8, 9, 10, 11, etc., where chromosome-formation has begun). It can always be distinguished from the chromatin granules by its luster and small spherical form. It takes no part in spireme-formation, but remains a separate element (Figs. 6 and 7). At the begin-

ning of nuclear activity it generally lies in that half of the nucleus which is nearest the sphere, and often quite close to it. I have been unable to trace it further than this, when it disappears. At about the same time — *i.e.*, at the beginning of chromosome-formation — the nuclear membrane, lying just below the sphere, shows distinct undulations or wrinkles, while it is intact and uniform at all other points. These can be seen only in sections, but I have found them in all sections of nuclei in this stage (Figs. 32–36), while in sections of nuclei in resting stages the membrane is as smooth at this point as at all others. At the points where the undulations appear, spaces are formed by the elevation of the membrane. These spaces are, as a rule, free from the basichromatin and oxychromatin of the nucleus (Figs. 32 and 33). In numerous cases, however, two minute, deeply staining granules were found in the spaces thus formed. The granules were always found in pairs, and in many instances no other granules could be seen (Figs. 32–34). In other cases, and, I am obliged to admit, in the majority of cases, other granules were found. The latter always had the appearance of chromatin, and it is not surprising that chromatin granules should be found here, for the nucleus at this stage is full of them. The two granules in question, however, have a distinct brilliancy, or luster, and usually have a definite position in relation to the elevations of the membranes (Figs. 32 and 33). Fig. 34 represents a section where the two granules lie just outside of the nuclear margin, which is distinctly indented at this point, and where the nuclear membrane could scarcely be distinguished. They are not in the substance of the sphere, but lie in a hyaline space between it and the nucleus. A similar stage is represented in Fig. 35. In this case only two other granules were visible, and these are represented on the left of the figure near the membrane. They are similar in form and appearance to other cytoplasmic granules found in the protoplasm of *Noctiluca*. Finally, in Fig. 36, the two granules lie in the sphere just outside of the nuclear membrane and the latter is rapidly assuming its regular contour. Other granules are pictured in the cytoplasm and in the sphere.

R. Hertwig ('96) doubts Ishikawa's account of the centrosome in *Noctiluca*, and, in accordance with his theory regarding this body, he would probably consider the sphere in *Noctiluca* as an enlarged centrosome. He also regards the centrosphere in the sea-urchin egg as an enlarged centrosome, in opposition to Boveri ('95), Hill ('95), and Kostanecki ('96), who have found centrosomes within it.[1] From his experiments with strychnine on developing eggs he also concludes that the centrosome may have the form of pole-plates, as in some Protozoa. He supposes the centrosome to be, originally, an intra-nuclear structure (as in *Euglena*, *Spirochona*, etc.), which becomes extra-nuclear in *Noctiluca*, *Paramoeba*, and the majority of Metazoa. The intra-nuclear pole-plates of most Protozoa, the sphere in *Noctiluca* and *Paramoeba*, and the sphere in Echinoderm eggs are, therefore, regarded by Hertwig as homologous structures, each of which he would call the centrosome. At first sight this is a most plausible theory, but many indubitable facts stand in its way. Boveri, Hill, and Kostanecki have found centrosomes in the sphere of sea-urchin eggs; Balbiani ('95) has described less substantially a centrosome in the pole-plate of *Spirochona;* and Ishikawa demonstrated the centrosome within the sphere of *Noctiluca*, an observation which I can fully confirm.[2]

As there is no trace of the centrosome in the sphere during nuclear rest, the conclusion is apparent that its presence here during nuclear activity can be accounted for either by formation *de novo* or by migration from some other part of the cell. This alternative involves a question in cellular biology which is far from settled — *viz.*, is the centrosome a permanent element of the cell?

In the first place, a number of investigators, especially among botanists, deny entirely the presence of a centrosome in certain dividing cells (Strasburger ('97), Osterhout ('97), Mottier ('97), etc.), while the remarkable observation made by Juel ('97) seems to indicate that the centrosome is not necessary in the formation of a spindle-figure. Juel found that a single chromosome was parted from its fellows and formed a

[1] Wilson also ('97) finds unmistakable centrosomes in *Toxopneustes* and *Arbacia*, which are derived from the middlepiece of the spermatozoa.

[2] See appendix, p. 49.

separate small cell around itself. It then passed into the resting stage, from which it emerged to form a mitotic-figure in which all of the elements, save centrosomes, were present. In the second place, a number of observers have maintained that, for each mitosis, the centrosome arises *de novo*, as in the well-known theories of Bürger ('92), Watasé ('93), Reinke ('94), and others. The results which Hertwig ('95) and Morgan ('96) obtained by treating the cell with dilute poisons, etc., are evidence in the same direction. Foot ('97) also holds that the centrosome in the first cleavage-spindle of *Allolobophora foetida* is the physiological effect of the spermatozoön upon the cytoplasm of the egg, and not a permanent morphological element brought in by the spermatozoön. To this list must be added a great number of observers who have followed the history of the centrosome up to a certain point in cell changes, after which they found no further trace of it. Finally, many observers have followed the centrosome from one generation to another and maintain it to be a permanent cell organ in accordance with the original view of Boveri ('87) and Van Beneden ('87). Brauer ('93) and the majority of spermatologists have traced the centrosome from generation to generation, in some cases up to a certain definite structure in the mature spermatozoön (Moore ('93), Meves ('96), Hermann ('92), Calkins ('95)), and many observers have followed the centrosome in the same way in the cleavage of the egg (Boveri ('87), Van Beneden ('87), Henneguy ('91), Mead ('95),[1] Griffin ('96)[2]).

From this review of the subject it is plain that the question of permanency of the centrosome has no immediate prospect of settlement. Repeated examinations of my sections, the evidence of which I have given above, I believe, justifies the conclusion, in a provisional sense at least, that the centrosome of *Noctiluca* is a permanent element ; that it exists during the

[1] Mead later ('97) comes to quite an opposite view. He says: "The foregoing observations convince me that the asters and centrosomes in the *Chaetopterus* ovum arise by modification of the cytoplasmic reticulum" (p. 394).

[2] Wilson ('97) has traced the sperm-centrosome through the first cleavage and into the 2-celled stage in *Arbacia*. Contrary to the view of Doflein ('97), he finds that the centrosome is not represented by the entire middlepiece of the spermatozoön, but that it is contained within the middlepiece which is thrown off as a shell after the spermatozoön enters the egg.

resting stages as a deeply staining granule in the nucleus (see p. 7); that it divides and gives rise to the two granules whose history I have given; that these finally come to lie in the cytoplasmic sphere where they separate as the amphiaster is formed, one going to each daughter-sphere, where mantle-fibers connect them with the chromosomes.

The nuclear origin of the centrosome has been observed in a number of forms. Thus, in all Protozoa hitherto described, with the possible exception of *Euglypha*, *Noctiluca*, and *Paramoeba*, the centrosome, or its equivalent, is intra-nuclear in origin. In the Metazoa the well-known results which Brauer obtained in the case of *Ascaris megalocephala univalens* have not been duplicated for other forms, although a number of observers have been led to think that, as in *Ascaris*, the centrosome is intra-nuclear. Among these may be mentioned Balbiani ('93), Julin ('93), Mathews ('94), and Carnoy and Lebrun ('97). O. and R. Hertwig have long maintained that the centrosome is primitively intra-nuclear, having been differentiated originally from part of the nuclear substance. There is a very important difference, however, between the intra-nuclear centrosome of Metazoa and Protozoa. Both Brauer and Mathews, for example, describe the centrosome as surrounded by a sphere, whereas in *Noctiluca* the sphere lies permanently outside of the nucleus.[1] If my observations are correct, sphere and centrosome in *Noctiluca* must, therefore, have an independent origin. As already indicated, however, there are so many chances for error in tracing the centrosome in *Noctiluca* through resting stages that this conclusion must remain an open question until further research throws more light upon it.[2]

[1] Mathews's statement of this process is as follows: "At maturation the centrosomes are first accurately to be distinguished as two (at a very early stage apparently as one) deeply staining, small, but distinct and characteristic, granules lying side by side either in the nuclear membrane or immediately without it, and invariably on that part of the vesicle nearest to the surface of the egg. Occasionally one of the granules appears before the other and migrates some distance from the nucleus before the second appears. In cases where they both lie clearly outside of the nucleus, the nuclear membrane is invariably broken behind them" (p. 324).

[2] Except for its decided staining qualities this intra-nuclear body might be considered a nucleolus and so be used as evidence in support of views of Karsten

### *D.* The Mechanism of Mitosis in Noctiluca.

The peculiar type of mitosis in *Noctiluca* may perhaps throw some light on the mechanism of mitosis in general. Here the entire absence of astral rays passing from the sphere into the surrounding cytoplasm excludes all contractility hypotheses in so far as they are based upon active physiological contractility of these fibers (Van Beneden, Boveri, Flemming, Reinke, etc.), and the same remark applies to Heidenhain's view that mitosis is affected through elastic tension of the rays. Furthermore, there is no morphological evidence to show that division is affected by contraction of the mantle-fibers (Hermann), for, as shown above, these do not shorten and thicken, but remain the same throughout mitosis. The possibility that their substance is taken up by absorption into the sphere, as Wilson has suggested for *Toxopneustes* ('96), is removed by reason of two facts, *viz.*, the daughter-spheres do not enlarge in the anaphase (spore mitosis) and the mantle-fibers can always be traced to distinct points in the sphere, *i.e.*, to the centrosomes. It must be, therefore, that the separation of the daughter-chromosomes is caused either by an active divergence of the spheres, or by growth of the central-spindle fibers and the consequent passive separation of the spheres (Drüner). The former is improbable because of the entire absence of the antipodal cones or astral rays, leaving the second as the only mechanical hypothesis which agrees with the facts.

My conception of the process is, then, as follows: the central-spindle lying within the ring of chromosomes is advantageously placed for exerting the necessary dividing force. The nuclear membrane disappears and mantle-fibers connect the ends of the chromosomes with centrosomes in the spheres. The central-spindle elongates, causing separation of the spheres; the mantle-fibers, remaining firm, move with the spheres, dragging

('94), Lavdowsky ('94), Carnoy and Lebrun ('97), and others who regard the nucleolus as the seat of the centrosome. On the contrary, it is a body which much more resembles the so-called "nucleolus-centrosome" described by Balbiani in *Spirochona*, and should be, I think, placed with the latter structure as one of the primitive forms of the sphere. See appendix, p. 49.

the ends of the chromosomes with them. As the central-spindle becomes longer, the chromosomes are more and more separated, until finally the distal ends are separated and chromosome division is completed.

R. Hertwig ('95) has already pointed out that this view of the mechanics of mitosis is perfectly consistent with the facts of nuclear division in the Infusoria. No mechanical hypothesis, however, can fully explain the different phenomena involved in the mitosis of *Noctiluca*. The action of the central-spindle cannot explain the first elongation of the nucleus in the primary axis, nor the later ring-form. Neither does it offer any explanation of the forces which cause the in-sinking of the central-spindle into the position of the secondary nuclear axis. The ultimate cause of these phenomena remains unexplained, and there seems to be little doubt that something deeper than mere mechanical force is necessary to explain mitotic activity. This is strikingly confirmed by Juel's observation on the isolated chromosome of *Hemerocallis fulva* and by Boveri's recent ('97) experiments on sea-urchin eggs in which he found that blastomeres are incapable of dividing when chromatin is absent.

## III. THE NUCLEAR RELATIONS OF NOCTILUCA TO METAZOA AND PROTOZOA.

### *A.* Relations to Metazoa.

The similarity of the mitotic-figures of *Noctiluca* to those of the Metazoa has already been indicated by Ishikawa. The comparison can now be carried still further. They agree in the following points: (1) the central-spindle fibers end in spheres which contain centrosomes; (2) the central-spindle occupies a position in the center of the nuclear plate; (3) the chromosomes lie freely around it without an intervening nuclear membrane; (4) the central-spindle fibers are not connected with the chromosomes; (5) mantle-fibers connect the chromosomes with the centrosomes; (6) the chromosomes in *Noctiluca*, like those of the Metazoa, are composed of granules which are at first separate, then unite to form a segmented spireme,

and after division again become disintegrated; (7) as in the Metazoa the chromosomes divide by longitudinal division; (8) the centrosomes, finally, are equivalent; they are the focal points of the mantle-fibers; they lie in the spheres during activity, and they divide during the anaphase in preparation for the ensuing division. There is some evidence that, as in *Ascaris megalocephala univalens* (Brauer), they come from the nucleus. In only one respect does the mitotic-figure in *Noctiluca* differ from that of the Metazoa — the nuclear membrane does not entirely disappear. In all other respects its description would answer for that of any ordinary metazoan mitotic-figure.

### *B.* Relations to Protozoa.

Mitosis in *Noctiluca* is so similar to that in Metazoa that in itself it throws little light on the origin of the process. The rapid increase of our knowledge of indirect division in the Protozoa has, however, made it possible to draw an accurate comparison between *Noctiluca* and other Protozoa in which the phenomena of mitosis appear in a still simpler form; and here we find some light on the possible origin of mitosis.

#### 1. *Origin of Chromosomes.*

In many primitive forms of protozoan nuclei, the chromatin is compressed into a single homogeneous sphere (*Uroglena*, *Dinobryon*, *Eudorina*, etc.), and with no indication of "achromatic" substances in the form of "ground-substances" or "nuclear-sap." In other forms of nuclei closely allied to these, the chromatin in the resting stage is similarly collected into a homogeneous sphere, but it lies imbedded in the nuclear ground-substance, the whole surrounded by a nuclear membrane (*Actinophrys sol*, Gruber ('**83**), *Heterophrys*, *Acanthocystis*, *Artodiscus*, etc., Penard ('**89**), and many Flagellates). Penard and Gruber found that in those cases where the chromatin forms a single mass it becomes divided into two, three, or more separate portions. Rhumbler ('**90**) agrees with Gruber and Penard in such an arrangement of the chromatin in Rhizopods and Heliozoa, and Wolters ('**91**), Labbè ('**97**), Clarke

('95), and others find similar results in the Sporozoa. Similarly Schultze ('66) described the nucleus of *Gromia* as variable ; one type containing very large granules ; another, granules of medium size ; and still a third type with very fine granules. Other observers on different forms of Protozoa have given similar descriptions.

From what we now know about the chromatin changes in Protozoa, it is probable that the different types of nuclei which, like *Gromia*, have been described for the same organism are, in reality, developmental stages in preparation for division. Gruber ('83) first showed that in *Actinosphaerium* disintegration of the central chromatin-mass is the earliest indication of mitosis. He found that it first divides into two portions, then into four, later into eight, and so on until a great number of minute chromatin elements fills the nucleus. The nuclear plate is then formed, and division of the nucleus ensues. This observation was confirmed by Hertwig ('84); and Brauer ('95) gave the same general result.[1]

In other Protozoa the chromatin is permanently in the form of small chromatin elements. This is the case in *Amoeba proteus*, *Amoeba binucleata*, and *Amoeba crystalligera*, *Euglena viridis*, *Cryptomonas*, and the macronuclei of the Infusoria. From Schaudinn's description ('94) the chromatin elements in *Amoeba crystalligera* do not change during mitosis but are simply separated into equal parts by nuclear division. Similar results were obtained by Blockmann ('94) and Keuten ('95) in *Euglena viridis*.

According to the latter's account, the nucleus of this flagellate consists, as in *Amoeba crystalligera*, of a peripheral ring of elongate chromatin bodies, and a central "nucleolus." In division the nucleolus first elongates while the chromatin is arranged in radial lines. Elongation of the nucleolus continues until the connecting-piece is reduced to a thin fiber. The chromatin is not formed into chromosomes, but is divided into two equal masses,

[1] Brauer's account of the resting nucleus differs in detail from that of Hertwig and Gruber. He showed the nucleus to be similar to that of a metazoan cell, consisting of chromatin in the form of a reticulum and "achromatin" in the form of linin. I have examined many hundred nuclei of *Actinosphaerium* in the vegetative state and after fixation with sublimate acetic and Hermann's fluid, but in none of them could I find the mononucleolate nucleus described by Gruber and Hertwig. The chromatin reticulum was invariably present with from two to four net knots.

and the daughter-chromatin elements gather around the daughter-nucleoli, with connecting or "Zwischenfaden" elements, which form parallel lines from pole to pole. These give the striated appearance which is so characteristic of protozoan mitosis. The daughter-nucleoli finally become totally separated, and the chromatin, which has become massed at the distal ends of the striae, but which still consists of separate units, forms two clumps around them.

In these forms the chromatin appears to be perpetually ready for nuclear division, and the many elements never fuse to form a homogeneous chromatin-mass. Brauer ('95) in describing the formation of the chromatin elements carried the analysis a step further in *Actinosphaerium*.

He found that, as in the metazoan nucleus, the first stage in division is the disappearance of the net knots, the reticulum becoming more distinct. The latter then disintegrates and the nucleus is filled with minute chromatin elements. "Auf einem etwas späteren Stadium ist der ganze Kernraum mit isolirten Körnen, den Chromosomen, erfüllt, deren Zahl nicht zu bestimmen ist" (p. 203). These are not chromosomes, as Brauer states, but elements which fuse later to form the chromosomes extending across the middle of the nucleus. They are next transversely divided, no longitudinal division taking place. It is probable that such an arrangement of the chromatin in the nuclear plate of *Actinosphaerium* represents a primitive stage in chromosome-formation.

The chromatin in metazoan nuclei passes through a similar stage before each mitosis, and similar elements are welded together to form chromosomes of definite shape and size for each species. A long step in the direction of this metazoan condition is taken by *Noctiluca*, where chromatin elements are formed as in *Actinosphaerium*, but become more compactly fused to form chromosomes of a certain distinct character, and where these chromosomes are divided longitudinally.[1]

[1] An entirely opposite view of the significance of chromosomes has been maintained by Mitrophanow. According to him, the chromatin in the nucleus of *Collozoum* is a single compact mass: "la chromatine, en forme d'une petite masse arrondie, et l'achromatine, que a l'aspect de deux appendices coniques" (p. 625). He insists that division here is a simplification of mitosis, which, "deviendra claire, si nous considérons la masse de chromatine comme une chromosome unique" (p. 626). This interpretation stands alone in the literature of protozoan mitosis, and instead of simplifying the problem makes it more complex. Mitrophanow's material was fixed with nitric acid and stained with an aqueous solution of safranin, and it seems probable, therefore, that a better technique will give quite different results in *Collozoum*.

A spireme-stage is wanting in the prophase of *Actinosphaerium*, and I have been unable to demonstrate a typical spireme in *Noctiluca*, although, as previously pointed out, the chromatin passes through a stage which simulates the metazoan spireme (Figs. 6, 7, and 12). There are, however, certain Protozoa in which a true spireme seems to occur. Karawaiew ('95) describes spireme-formation in the Radiolarian *Aulocantha scolymantha*. In the resting stage, this nucleus has a large "spongy" nucleolus, consisting of a dense central mass with numerous branches. When preparing for division the "spongy" mass breaks up into threads, until finally the entire chromatin-mass becomes a tightly wound spireme. Sooner or later the spireme-threads undergo a longitudinal division, at the same time becoming granular. Karawaiew saw no nuclear plate, the next stage after the spireme being a late anaphase in which two striped regions were found at the poles. Each stripe consisted of a row of chromatin-granules. The account is by no means complete, and it is probable that more careful examination will show a nuclear plate.

Our acquaintance with the chromatin-changes in the micronuclei of ciliates is more satisfactory. The exceedingly dense structure of the micronuclei has led Hertwig and Gruber to class them as "massive nuclei." During division they swell considerably, a change invariably preceded by a spireme-formation (Maupas ('89) and Bütschli ('76) ). The spireme gives rise to granular chromatin-threads which as in *Actinosphaerium*, become thicker in the central portion, where they are finally divided by transverse division.

In the great nuclei of Dinoflagellates also, Bütschli ('85) and Lauterborn ('95) have shown that the chromatin-reticulum becomes "increased in size" until a "much-twisted Knäuel" results. After this the chromatin becomes arranged in more or less regular parallel fibers which are divided transversely. Zacharias describes for the same form a much more complex process of mitosis, but his results are denied by Lauterborn and others.

A much more complicated mitosis, including spireme and chromosome-formation, was described by Pfitzner ('86) in the

case of *Opalina ranarum*. Schewiakoff ('88) gives a somewhat similar description of mitosis in *Euglypha alveolata*.

> Here the chromatin network of the nucleus gradually becomes thicker, especially in the region of the net knots, until a coarse spireme appears. Threads composed of many small particles — the "chromatin granules" of Pfitzner — are finally formed from the spireme; these are ragged at first, but become homogeneous and smooth, after which they arrange themselves around the periphery of the nucleus. The threads then shorten and become bent into loops, the angle being turned towards the axis of the spindle. At this stage each chromatin-loop is divided longitudinally and the daughter-chromosomes separate. After the formation of a daughter-spireme the nucleus returns to the reticular state.

From this examination of the different changes undergone by the chromatin in the various forms, it is possible to get an idea of the probable development of chromosomes, although the many gaps in the series and the often incomplete observations make it far from conclusive. The most primitive structure would seem to be the mononucleolate forms in which the nucleus has no "cell sap," and where division is possibly "amitotic." An advance is shown in forms where the mononucleolate chromatin-mass breaks up into smaller elements during division, as in some Rhizopods and Heliozoa, and in many Protozoa just before spore-formation. A still higher type is shown in forms where the chromatin exists permanently in the form of small granules (many *Amoebae*, *Euglena*, etc.). In *Euglena* and *Amoeba* these chromatin-granules do not fuse to form definite structures (chromosomes); they simply separate half from half, and they are clearly equivalent to the minute elements, which in other cases are formed by the breaking down of chromatin-masses. The aggregation of chromatin elements into more or less definite chromosomes is shown in a primitive way in *Actinosphaerium* and the micronuclei of Infusoria. In *Noctiluca* the aggregates become more compact, definite, and chromosome-like, while for the first time they are divided longitudinally. A still more metazoan-like chromosome structure is shown by *Euglypha alveolata*, where the chromatin elements are not distributed throughout the nucleus, but unite at once to form a tightly wound thread — the spireme — from which the chromosomes are later derived by transverse division.

These chromosomes, as in *Noctiluca*, divide longitudinally, and daughter-spiremes are formed, after which the chromatin passes again into the resting reticulum. In *Euglypha*, therefore, the chromatin-changes seem to be practically the same as in the Metazoa, and the elements are even more highly differentiated than in *Noctiluca*, for the latter has no chromatin-reticulum nor definite spireme. The aggregation of the chromatin elements after division to form karyosomes in *Noctiluca* is another indication of the more primitive nature of this form. *Noctiluca* and *Euglypha*, therefore, may justly be considered as connecting links, so far as chromatin is concerned, between the conditions in Metazoa and the simplest Protozoa.

### 2. *Origin of Centrosome and Sphere.*

The origin of the metazoan centrosome and attraction-sphere from simpler elements in Protozoa has been the subject of a number of interesting theories. The most recent and the most important of these have been maintained by Heidenhain ('94), Lauterborn ('96), and Hertwig ('96). According to Heidenhain the "central-spindle," as described by Hermann ('91), is identical with the spindle formed by the micronucleus of the Infusoria after the latter has undergone a loss of chromatin and has acquired a differentiated center — the centrosome. He regards the nucleus of the metazoan cell as derived from the infusorian macronucleus, while the mantle-fibers are new formations. Not only does he compare the nucleus and centrosome with the macro- and micronuclei of Infusoria, but he even makes this comparison the basis of a theory in which he derives the Metazoa from the Infusoria. The comparison of centrosome in Metazoa with the micronucleus is not original with Heidenhain. Bütschli ('91) had already proposed it, and Hertwig and Lauterborn had adopted the same view. Lauterborn's recent attempt to derive the "achromatic" structures of Metazoa from elements in the Protozoa is even more ingenious than that of Heidenhain. The essential difference between the two theories is that in the one case (Heidenhain) the centrosome has been derived directly from the nucleus, while in the other (Lauterborn),

centrosome and micronucleus are supposed to have had a common origin. Lauterborn thinks that centrosome and micronucleus may have had a common ancestor in one of the nuclei of some primitive binucleated Protozoön, and that intermediate stages are represented by certain existing Protozoa. Schaudinn's *Paramoeba* furnishes an hypothetical early stage in differentiation of the centrosome, which there is represented by the Nebenkörper. *Noctiluca* and the diatoms represent a more advanced stage toward the metazoan centrosome. On the other hand, the micronucleus of the Infusoria represents a differentiation of the same primitive nucleus in a different direction.

Hertwig's theory deals more specifically with the development of the "achromatic" parts of the mitotic figure. He expresses it in one paragraph as follows: "Bei den Protozoen finden wir alle Uebergänge von der gewöhnlichen Durchschnürung des Kerns bis zu komplicirten Karyokinesen. In vielen Fällen—z. B. den Hauptkernen der Infusorien—ist unzweifelhaft während der Theilung ein achromatisches, dicht mit Chromatinkörnchen beladenes Netzwerk allein der Sitz der treibenden Kräfte; es bilden sich weder Spindelfasern noch Polplatten. Bei *Ceratium hirundinella* ordnet sich das achromatische Kernnetz schon zu Spindelfasern an, auf denen die Chromatinkörnchen gleiten, um auf die Tochterkerne vertheilt zu werden. Einen weiteren Fortschritt macht *Spirochona* durch die Entwickelung von Polplatten. Unzweifelhafte Karyokinesen endlich treffen wir bei *Actinosphaerium*, *Actinophrys*, *Amoeba binucleata* den Nebenkernen der Infusorien. Bei *Paramoeba Eilhardi* und *Noctiluca* scheint sogar der letzte Schritt der Vervollkommnung, die Ausbildung von Centrosomen, gemacht zu werden. Wir würden daher drei verschiedene Ausbildungsstufen in der Entwickelung der Centrosomen aufstellen können: (1) Die achromatische Substanz ist im ruhenden Kern zwar noch gleichmässig vertheilt, liefert aber während der Theilung Polplatten als Aequivalente von Centrosomen. (2) Die achromatische Substanz ist dauernd zu einem intra-nucleären Centrosoma umgebildet. (3) Sie ist zur Bildung eines extra-nucleären Centrosoma aus dem Kern herausgetreten" (pp. 77, 78).

While agreeing with Hertwig's general conception of "achromatic" structures in Protozoa, I believe that he has left out of account a number of facts which have an important bearing on the general problem. The "sphere" in *Noctiluca* is not the centrosome and must be distinguished from it; the centrosome in *Actinosphaerium* (Brauer) cannot be the same as the "pole-plate," and the "intra-nuclear" granule described by Schaudinn in *Amoeba*, and the so-called "centrosome" described by Balbiani must have some significance apart from the "achromatic" structures which accompany them. If we take these various structures into consideration, the problem becomes much more complex, and the possible differentiation of sphere (archoplasm) and centrosome must be sought for much earlier in phylogeny.

The most primitive nuclei in which a differentiated "achromatic" body occurs are found in *Euglena viridis* and *Amoeba crystalligera*. In both of these the nucleus consists of a nucleolus-like body with surrounding chromatin. Keuten ('95) describes this body, which up to this time had been called a "nucleolus,"[1] as similar to a nucleolus in its staining reactions, but as playing a different rôle in nuclear division. He therefore calls it a "nucleolus-centrosome."

My own observations on *Euglena viridis*, and on a species of *Cryptomonas* which has a similar central body, confirm every stage given by Keuten, but I give a different interpretation to the staining reactions of this so-called "nucleolus-centrosome." The color reactions which he describes for this body are not those of a centrosome. It becomes "orangegelb" when stained with Orange G; with carmine solution it stains more intensely than the chromatin, while with haematoxylin it takes only a faint stain. In my preparations it takes a haematoxylin stain, remaining black or blue when the rest of the cell is stained with Orange G.

These reactions are characteristic of archoplasm or of the centrosphere, and for this reason, if for no other, the name "nucleolus-centrosome" is not entirely appropriate. Instead of comparing it with the centrosome of the Metazoa, it would be much more accurate to compare it with the sphere in *Noctiluca* or with the pole-plates of other Protozoa. A true centrosome has not been found in it.

[1] Considerable confusion has arisen because of the indiscriminate use of the term "nucleolus" in connection with protozoan nuclei. It has been applied to the chromatin-masses (karyosomes) and to various "achromatic" structures.

A similar central nuclear body has been described by Schaudinn in *Amoeba crystalligera*. With high powers he was able to make out a certain alveolar ("wäbige") structure of the "nucleolus" ("achromatic body"), and, in addition, he occasionally found in it a granule or granules which stained with chromatin dyes. He included all of these granules in the nucleolus. The chromatin undergoes no preparatory changes before division, and the nucleus divides first, the deeply staining granules having meantime disappeared.

Schaudinn considers this a confirmation of F. E. Schultze's earlier description of the division of *Amoeba* as amitotic, although he seems to realize the significance of the nucleolus when he says: "Der als Nucleolus bezeichnete Theil des Kerns scheint bei Durchschnürung des Kerns, wie Fig. II *b* u. III *b* zeigen, die Hauptrolle zu spielen" (p. 1035). The presence of granules within the "nucleolus" is interesting, and may be taken as a possible indication of an early stage in the differentiation of a centrosome.

The intra-nuclear "achromatic body" plays a more important rôle in the nuclei of *Euglypha*, *Spirochona*, and *Kentrochona*. In the former the bodies at the poles of the spindle are considered by Schewiakoff the same as the "Polkörperchen" (centrosomes) of metazoan nuclei. Their history is not clearly made out, but his description seems to indicate a nuclear origin, though he himself draws a different conclusion. His account is as follows: "The polar cytoplasm develops radial rays ('Polstralen') which converge at the 'Polkörperchen' (pole-plate) in a small invagination of the nuclear membrane. At the same time spindle-fibers make their appearance inside of the nucleus, and he concludes, therefore, that the 'Polkörperchen' is derived from the pole-rays by the coalescence of the cyto-microsomes lying in them. There is no mistaking Schewiakoff's meaning; the pole-bodies are derived from the cytoplasmic granules. There are, however, some significant features in his account of the division which throw considerable doubt on this inference. In the first place, at the time when the spireme is well formed, and just before the formation of the 'Polkörperchen' the so-called 'nucleolus' disappears.

There is, in reality, no occasion for using it at all, for the meaning is much better expressed by the terms "chromatic body" (or karyosome) and "achromatic body."

In the second place, during the anaphase 'the chromatin-loops become reduced to thick threads and the 'Polkörperchen' is redrawn into the nucleus.' Finally, after the stage just described, the chromatin regains first its coarse and then its fine mesh-like structure, and the 'nucleolus' reappears in the nucleus. If the 'Polkörperchen' is withdrawn into the nucleus, and if the nucleolus reappears shortly afterwards, it is probable that the 'nucleolus' is derived from the pole-bodies. Also, if the 'nucleolus' is thus derived from the 'Polkörperchen,' and if it disappears when the new pole-bodies are formed, an equally possible inference is that the pole-bodies are actually derived from the 'nucleolus.'" There is reason, therefore, to believe that in *Euglypha*, also, the "achromatic bodies" are intranuclear in origin.

The nuclear division of *Spirochona* was first described by Hertwig ('77). Since then it has been described by a number of observers, Balbiani ('95) being the most recent. Hertwig describes the resting-nucleus as consisting distinctly of two parts, one finely granular, and the other homogeneous in structure. Balbiani ('95) has, in the main, confirmed Hertwig's description of the *Spirochona* resting-nucleus. He finds, however, that the two parts of the nucleus are separated by a fissure or "fente," which he thinks is the "Kernspalt" of the ciliate macronuclei. The "homogeneous" part, which undoubtedly corresponds to the "achromatic body" of other Protozoa, is, therefore, within the nuclear membrane, but, as in *Noctiluca* and *Paramoeba*, it is completely separated from the chromatin. Hertwig describes a small central granule in the homogeneous part, while Balbiani's description shows that other structures of a fibrous nature are also present before this body appears. He finds in stained preparations that the achromatic part (which appears homogeneous during life) contains numerous short fibers which form a network. These fibers do not stain with ordinary chromatin dyes and become arranged in a certain definite manner about the "nucleolus" (central granule in the "homogeneous" or achromatic body), which appears later.[1]

[1] "La partie dite homogène du noyau ne merite pas non plus cette qualification dans le noyau fixé par les reactifs. Elle aussi présente un continu filamenteux

The exact history of the achromatic body of *Spirochona* is not given either by Hertwig or Balbiani. The former says that at times of division the homogeneous part enlarges, and a small central granule, which he calls the "nucleolus," appears. This gradually changes by sending out amoeboid processes, after which it becomes more and more indistinct until it can no longer be made out. The granular chromatic portion breaks into smaller pieces until the entire nucleus appears homogeneous. After this two masses of homogeneous substance, which he calls the "end plates," appear at the two extremities, and these he regards as the same substance as the original homogeneous portion. Balbiani also thinks that the "end plates" or pole-plates (Calottes) are the same as the homogeneous part of the resting-nucleus.[1]

In all the larger *Spirochona* nuclei the homogeneous part (*i.e.*, the "achromatic body") encloses that granule which Balbiani with Hertwig calls the "nucleolus." In smaller nuclei, on the other hand, an analogous body is found in the granular and not in the homogeneous part. Hertwig shows no connection between these two granules, but claims that the "nucleolus of the homogeneous part is the homologue of the metazoan nucleolus." Plate ('86) gives a somewhat different account, and admits a possible identity of the two. The "nucleolus" of the homogeneous part, he thinks, is formed from particles of chromatin which penetrate, while in solution, from the granular into the homogeneous portion, and there reform into the solid refringent corpuscle. Bütschli regards the corpuscle as a condensation of the chromatin-reticulum of

comme la partie granuleuse, mais les filaments sont plus courts, plus fins, beaucoup moins nombreux, et au lieu d'être placés parallelment les uns aux autres, ils s'entrecroissent diversement dans la substance homogène et transparente (suc nucleaire) dans laquelle ils sont plongés. De plus ils ne se colorent pas, ou faiblement, par les colorants de la chromatine, notamment le vert de methyle, et se montrent des lors comme formes d'achromatine. Cette structure de la partie homogène ne s'observe que dans les noyaux qui ne contiennent pas encore un nucleole (centrosome) ou dans ceux où le nucleole se trouve encore dans la partie granuleuse, lorsqu'il est parvenu dans la partie homogène les filaments achromatiques prennent une autre disposition que nous decriron par la suite" (p. 248).

[1] "Nous pouvons donc conclurer à une identité morphologique et chimique complête entre les masses polaires du noyau en division et la partie achromatique du noyau au repos" (p. 292).

the homogeneous portion. Balbiani agrees with neither. He thinks that the "nucleolus" which is found in the granular part of the nucleus is not an initial but a final step in division (telophase), the corpuscle or "nucleolus" forming as follows: The ends of all the chromatin filaments converge at the pole during the telophase. These ends, which at first are closely pressed together, separate later, and a space or vacuole is made containing several granules. The latter collect together at a later stage to form a single corpuscle. This corpuscle is the "nucleolus," and from here it passes through the granular and into the homogeneous part of the nucleus, where it undergoes the changes so carefully described by both Hertwig and Balbiani.

The peculiar changes which this corpuscle undergoes led Balbiani to regard it, quite independently of the "pole-plates," as the "centrosome."

He says: "Le globule central participe à la fois des caractères d'un nucleole vrai, et d'un centrosome; comme nucleole, il disparait par resorption dans la substance achromatique au debut de la division, pour se regenerer chez les deux nouveux noyaux par les processus indiqué plus haut; comme centrosome il condense autour de lui la substance environnante son forme d'une petite sphere attractive intra-nucleaire, que ne passe pas du noyau dans le protoplasma pour y jouer le rôle d'un centrosome ordinaire pendant la division de la cellule" (p. 309). He denies Hertwig's homology of "end plates" to "Polkörperchen" or centrosomes, because they are nuclear in origin, whereas the latter are cytoplasmic, and he attempts to explain them "comme des accumulations, aux deux poles du noyau en division, de sa substance achromatique, et leur destinée est de ramener les noyaux nouveaux, au type normal du noyau au repos chez le Spirochone" — which explains nothing at all.

From Balbiani's description of the "achromatic" parts in *Spirochona* it is evident that this nucleus presents one achromatic element separated from the chromatin by a "fente," and similar to the sphere in *Noctiluca;* and a second element, — the so-called "centrosome" — which is derived from the chromatic portion of the nucleus. This body passes from the chromatin into the "achromatic" part of the nucleus, possibly in the same manner as the centrosome in *Noctiluca* is supposed to pass from the nucleus into the sphere. It cannot be identified with

the "nucleolus-centrosome" of *Euglena*, for the latter is "achromatic" and probably equivalent to the pole-plates of *Spirochona*.

A very similar description has been given in the case of another parasitic ciliate, *Kentrochona Nebaliae*. According to Römpel's ('94) description, the nucleus of *Kentrochona* develops at one pole an "achromatic" mass, which extends outwards and bears two centrosomes at its extremity. After this mass is well developed a similar structure appears at the opposite pole.

Römpel then proceeds as follows: "Wie wird der Gegenpol gebildet? Genauer, in welchem Lageverhältniss stehen Chromatin und Kernspindel (achromatic portion) während der Ausbildung des Gegenpols? Nach den vorliegenden Präparaten dürften überhaupt nur zwei Möglichkeiten in Betracht kommen. Entweder wird das von Anfang an ring- oder cylinderförmige Chromatin von der Kernspindel central durchbohrt, und diese wird so zur Achse des Chromatinhohlcylinders, oder die Kernspindel zieht sich unter dem ventral eingebuchteten Chromatinhohlcylinder her." Römpel's Fig. 4 *e* represents the latter alternative, which certainly suggests the action of the sphere in *Noctiluca;* for in *Kentrochona* the "achromatic Kernspindel," like the "central-spindle" of *Noctiluca*, is apparently sunk in the nucleus and stretches from pole to pole.

Römpel's account of the division of *Kentrochona* is very faulty, and the different stages described are separated by wide gaps. His "centrosomes," too, have been questioned by Hertwig ('96), Balbiani ('94), Flemming ('94), and others, most of whom think he has mistaken micronuclei for centrosomes. Their criticisms are upheld by Doflein ('96, '97), who has recently reëxamined mitosis in *Kentrochona*. According to him the process of division is very similar to that of *Spirochona*.

"Die Theilung des Hauptkerns stammt in den grossen Zügen mit derjenigen bei *Spirochona* überein, wie sie besonders eingehend R. Hertwig und Balbiani geschildert haben" (p. 363). The chromatin is sharply defined against the achromatin which "in seitlicher Ansicht erscheint sie als Anlage der Polplatte" (p. 364).

Doflein mentions a distinct granule in the pole-plate during the metaphase, which must be analogous to the so-called "nucleolus" or "centrosome" of Spirochona according to Balbiani.

In *Actinosphaerium* the centrosome has been described by only one observer, Brauer ('94), and its presence has been since denied by Hertwig ('96). Here as elsewhere, however, one positive observation by a good authority must bear more weight than a number of negative statements. Both Hertwig and Brauer are agreed that the pole-plates in *Actinosphaerium* are derived from the intra-nuclear "achromatic" body of the resting nucleus. Brauer describes an accumulation of cytoplasm outside of the pole-plates which he calls the cytoplasmic "Kegel." The nuclear membrane is never lost at any point, nor is there any apparent connection between the protoplasmic "Kegel" and the inside of the nucleus, for they are separated by the pole-plates which "machen den Eindruck, als wenn es Verdickungen der Membran wären, die stets während des ganzen Kerntheilungsprocesses erhalten bleibt" (p. 204). The cytoplasmic accumulation appears only during mitosis. "Ausserhalb des Kernes an seinen Polen beginnt sich schon, wenn die ersten Veränderungen im Kern erkennbar werden, feinkörniges Protoplasma anzusammeln" (p. 204). In certain cases centrosome and radiations were seen in this mass, and from Brauer's figures of these stages it appears that the pole-plates at this time are either absent (Figs. 44, 46) or else much reduced (Fig. 45). Brauer is inclined to think that the centrosome is within the pole-plate during prophase and metaphase, and that it finds its way thence into the protoplasm during the anaphase. "So scheint mir der Schluss unabweisbar, dass das Centrosom vorher nicht im Protoplasma ausserhalb des Kernes, wie die Figuren es zeigen, gelegen haben kann" (p. 207).

Thus in *Actinosphaerium* the substance of the pole-plates, or a part at least, becomes extra-nuclear for a short time. In *Paramoeba* (Schaudinn, '96) and in *Noctiluca* it is permanently so. Schaudinn describes it in *Paramoeba* as follows: "Dicht neben dem Kern liegt stets das bereits zu Anfang erwähnte, stark lichtbrechende, scharf construirte Gebilde. Ich will dasselbe zunächst mit einem ganz indifferenten Namen, etwa als 'Nebenkörper' bezeichnen. Bei den kleinsten Amoeben ist es kugelig und ungefähr von derselben Grösse wie der Kern." During spore-formation, *pari passu* with a progressive

division of the nucleus, this "Nebenkörper" breaks up into smaller masses, each of which becomes associated with one of the daughter-nuclei. Here it forms a central-spindle, and the spore-nuclei divide by mitosis, in the same way, apparently, as in *Noctiluca*. A centrosome is not described.

A review of the foregoing facts shows that different forms of Protozoa present a nearly complete series in which may be traced the possible development of the complex extra-nuclear centrosome and sphere from an intra-nuclear "achromatic" body. This, — the homologue of the attraction-sphere, — apparently, first appears as an axial nuclear rod in *Amoeba crystalligera* and *Euglena viridis*. In *Spirochona*, *Kentrochona*, and the micronuclei of Infusoria it is seen as aggregations of "achromatin" — pole-plates — at the spindle poles. In *Actinosphaerium* the pole-plates remain intra-nuclear for the greater part of mitosis, but according to Brauer a portion of them at least becomes extra-nuclear for a brief period ; and during this period spindle-fibers are formed. The "achromatic" body remains extra-nuclear in *Paramoeba*, in *Noctiluca*, and in the majority of Metazoa. In *Noctiluca* and the Metazoa a portion of the "achromatic" body, — the central-spindle, — although extra-nuclear, comes to lie during mitosis in the center of the nuclear plate, where it occupies the same position, morphologically, that it occupies in *Amoeba crystalligera* or *Euglena viridis*.

The centrosome is not so easily traced, and it may be found that in the majority of Protozoa the intra-nuclear so-called "centrosome" is quite a different structure from the centrosome of the Metazoa, although even here the centrosome has been traced to an intra-nuclear position by many observers. Its nuclear origin in Protozoa is made out by Balbiani in *Spirochona* and by Doflein in *Kentrochona*, while Schaudinn describes a body in *Amoeba crystalligera* which must be homologous. In one case at least — *Spirochona* (Balbiani) — its direct morphological connection with the chromatin has been traced, although not conclusively.[1]

[1] In this case the centrosome relation can be questioned on account of the place of origin of the supposed centrosome. In most cases the centrosome is found in the region of the spindle poles, but in *Spirochona*, according to Balbiani,

In *Spirochona* the so-called "centrosome" passes from the nuclear chromatin into the achromatic body, which, however, still lies within the nuclear membrane. It is possibly nuclear in origin in *Actinosphaerium*, becoming extra-nuclear during the metaphase of division. It appears to be nuclear in origin in *Noctiluca*, but is found in the cytoplasm in the achromatic body in the early metaphase and remains permanently in the cytoplasm in spore-forming individuals. Finally it is permanently cytoplasmic in the Metazoa, although occasionally nuclear in origin even here (*Ascaris megalocephala univalens*).

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The important position that *Noctiluca* must hold in all theories of mitotic development has not hitherto been sufficiently emphasized. Much stress, indeed, has been laid on its similarity in mitosis to metazoan cells, but little has been done to show its relations to other Protozoa and the origin of its mitotic structures. When considered alone, *Noctiluca* can throw but little light on the origin of the complex elements of the metazoan mitotic-figure, but when considered in connection with other Protozoa the origin of its own elements is seen, and so, indirectly, the probable origin of the elements in Metazoa. *Noctiluca* thus holds an intermediate position in the probable development of mitosis, and the inference may be drawn that the origin of the mitotic elements in Metazoa is the same as in *Noctiluca*.

The position which *Noctiluca* holds in the development of chromatic structures has been sufficiently pointed out. It has been shown, first, that *Noctiluca* has probably the most primitive form of true chromosomes; and, second, that *Noctiluca* presents probably the most primitive ring-like arrangement of the chromosomes and nuclear plate around the central-spindle. In regard to the "achromatic" structures the position of *Noctiluca* is not quite so definite, and to be understood must be considered in connection with lower forms. It has been main-

it originates from chromatin in the region of the "Zwischenkörper." Further work on this questionable structure must be done before its proper position can be determined; that it is an important element in the cell and has a rôle to play in nuclear division is established beyond question by the independent observations of Hertwig, Plate, Bütschli, and Balbiani.

tained by numerous observers that the so-called "achromatic structures" of the mitotic-figure are primitively nuclear in origin. (See O. and R. Hertwig ('93 and '96), Heidenhain ('94), etc.) Furthermore, in the various kinds of Protozoa which have been carefully studied the "achromatic" portions of the mitotic-figures are developed from permanent, definite bodies which are independent of the chromatin, although contained within the cell-nucleus. As shown elsewhere in this paper, two apparently similar elements of the metazoan cell have been described, either or perhaps both of which may be comparable to these intra-nuclear achromatic bodies in Protozoa. The first of these is the "archoplasm" of Boveri; the second is the substance which forms the "centrodesmus" of Heidenhain, which is described as a specific substance of the cell, as playing a certain definite rôle in both resting and active phases, and as distinct from the centrosome, from the nucleus, and from the cytoplasm. Whether we consider this substance in the form of "centrodesmus" or as archoplasm it appears, therefore, that the cells of some Metazoa have a permanent specific substance which may be considered the homologue of the "achromatic body" of Protozoa.

Boveri does not consider a nuclear origin contrary to the conception of archoplasm, and with many others regards the "achromatin" in many protozoan nuclei as archoplasm ('97). He even holds it possible that there may be nuclei in Metazoa which show a return to this primitive condition. Heidenhain does not mention the derivation of the "centrodesmus" from any other structure in more primitive forms. The only standards of identification are: its relation to the centrosome, the formation of the spindle from its substance, and its permanency. These same points may be used equally well for determining the more primitive forms of archoplasm.

In *Noctiluca* the so-called "sphere" surrounds the centrosome at the period when the latter is unmistakable. Here, also, the central-spindle is formed from the substance of the sphere, and the latter is permanent in the cell, persisting as a specific substance throughout all stages and quite distinct from the cytoplasmic reticulum. The sphere in *Noctiluca*, therefore,

appears to be equivalent to the archoplasm of Boveri and the centrodesmus of Heidenhain. In the more primitive forms, unfortunately, the behavior of the intra-nuclear "achromatin" during division is not well enough known to warrant definite conclusions, although the fragmentary evidence which has been gathered from various sources is sufficient to show, I believe, that structures possessing all of the attributes of archoplasm are present in the various forms. Its history is well known, indeed, in *Euglena*, but here we are confronted with the assumption, by Keuten and others, that the intra-nuclear element is a centrosome or its equivalent. There is reason to believe, however, that the two poles of this achromatic body represent the pole-plates of other Protozoa, and that the connecting rod represents the central-spindle. If this hypothesis is correct, the achromatic body in the nucleus of *Euglena* must be considered equivalent to the sphere in *Noctiluca* and to the archoplasm of Boveri or the centrodesmus of Heidenhain.

With our present knowledge it is impossible to go farther back than *Euglena* for the development of archoplasm, and the conclusion which may finally be drawn from our present knowledge of this difficult question seems to be that primarily there was a specific substance of the cell (archoplasm in Boveri's sense) connected in some way with the mechanism of cell division, and forming a definite intra-nuclear body (*Euglena*). Secondly, that this body became permanently extra-nuclear, but still connected with nuclear division (*Noctiluca*, and Metazoa with "centrodesmus"), and finally that it became lost in the cell and indistinguishable from the cytoplasmic reticulum (cells without archoplasm or centrodesmus, most egg cells).

The close similarity of mitosis in *Noctiluca* and Metazoa does not necessarily indicate any phylogenetic connection. Nor do the various Protozoa, which in this analysis are necessarily brought together, show phylogenetic characters. We are at present unable to develop any phylogenetic theory from the facts of nuclear division. All that can be maintained is that mitosis, in its many complicated phases, may have passed through stages of development which are to-day represented by many different and unallied types of Protozoa.

## IV. SUMMARY OF OBSERVATIONS.

1. The resting nuclei of *Noctiluca miliaris* are large, round, or oval structures, containing (*a*) chromatin in the form of karyosomes, and (*b*) "achromatin" in the form of large granules. It is enclosed by a nuclear membrane which persists in part throughout nuclear division.

2. A cytoplasmic substance, corresponding to the centrosphere of many metazoan cells, is invariably present. It is a permanent organ of the cell, often as large, or larger, than the nucleus; it divides to form an amphiaster, consisting of two asters with connecting mantle-fibers, the central-spindle.

3. During the division of the sphere the nucleus elongates and bends to form a figure like the letter C; the central-spindle sinks into the opening thus formed, and is finally almost enclosed by the nucleus.

4. As division progresses, the central-spindle becomes three or four times as long as it is in the metaphase.

5. The karyosomes break up, by repeated division, into innumerable chromatic elements. In some cases beaded fibers are formed by the linear arrangement of larger chromatin particles. These fibers are the only indication in *Noctiluca* of a spireme-stage. The chromatin elements begin to form the chromosomes, which, at first, are lines of single granules extending from the nuclear membrane on the side next the sphere towards the opposite side. At this stage they appear like radial-fibers extending around the central-spindle and forming a nearly closed ring. This incomplete ring of chromosomes is the nuclear plate.

6. The chromosomes next become thicker, especially at the ends next the central-spindle, and, probably, by the aggregation and fusion of the granules. This enlargement continues towards the opposite ends, until, finally, the chromosomes are of uniform thickness.

7. The chromosomes divide longitudinally and while lying in the nuclear plate. The halves then separate, beginning at the proximal ends.

8. While the chromosomes are forming, the nuclear membrane disappears from between the chromosomes and the central-spindle. This leaves the chromosomes in contact with the spindle-fibers.

9. The central-spindle fibers have no connection with the chromosomes, but pass without interruption from pole to pole. The chromosomes are connected with the spheres by another set of fibers, — the mantle-fibers, — which pass from centrosomes in the spheres to the ends of the chromosomes.

10. The nucleus, during the anaphase, elongates in the direction of the central-spindle. The chromosomes are pulled apart, the final division taking place at the distal end of each. As they separate still more, they form two sets of oppositely-directed striations in the nucleus, and the daughter-chromosomes again thicken at the proximal ends.

11. The nucleus, finally, divides in the center, often with a very large connecting-piece between the daughter-nuclei. The furrow is obliterated, the nuclear membrane reforms, and the nucleus rounds out. The sphere loses its densely compact appearance and becomes more expanded, although its granular structure is retained.

12. In spore-forming divisions the nuclei do not return to the resting state. The daughter-spheres divide and form secondary or tertiary, etc., amphiasters ; the daughter-chromosomes form the nuclear plate of the next mitosis without change ; and split again longitudinally. This process is repeated eight or nine times.

13. A centrosome is always found in the sphere during metaphase and anaphase stages as the focal point of the mantle-fibers, but is not found during resting stages. It divides in the early anaphase in anticipation of the next mitosis.

14. The centrosome, possibly, comes from the nucleus, where, during the resting stages, a small, deeply staining granule can be easily distinguished from the chromatin. This granule disappears during the early stages of chromosome-formation. At the same period the nuclear membrane, just below the sphere, shows distinct undulations, or wrinkles, which form a clear space below the membrane. In numerous

cases two distinct granules were seen in this space. These granules occupy various positions in relation to the membrane, and in some cases they were found outside of the nucleus and in the sphere. The inference is therefore drawn that the centrosome is a permanent cell organ, which is not found in the resting sphere, but in the nucleus, from whence it becomes extra-nuclear by a rupture in the nuclear membrane. The observations on this head are not conclusive, owing to the smallness of the objects and to the presence of many similar cytoplasmic microsomes.

COLUMBIA UNIVERSITY,
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## APPENDIX.

Since the above was written a number of forms have been carefully examined in the hope of finding Protozoa in which the archoplasm may be traced back to a type still more primitive than *Euglena viridis*. It was found that *Trachelomonas hispida*, *T. volvocina*, *Microglena punctifera*, and *Synura uvella* have nuclei similar to that of *Euglena viridis; i.e.*, with a central body and chromatin in the form of small granules. In another set of forms, including *Trachelomonas lagenella*, *T. hispida* (variety), and *Chilomonas cylindrica*, the chromatin is in the form of granules and, as in *Euglena*, surrounds a central body, but, unlike *Euglena*, there is no nuclear membrane. Finally, in a species of *Tetramitus*, not only is the nuclear membrane absent, but the granular chromatin is distributed throughout the cell, and only during division are the granules collected around a central body. This central body corresponds evidently to the Nebenkörper of the flagellate-stage of Schaudinn's *Paramoeba Eilhardi*, but in *Tetramitus*, except during cell division, it is cytoplasmic, no morphological nucleus being present. The relations of this body to the chromatin are the same as the relations of the sphere to the chromatin in *Noctiluca*, although the former is far more primitive because of the distributed nucleus (*cf.* Bacteria). It would seem, therefore, that archoplasm was originally cytoplasmic; that it attracts (?) the chromatin about it during cell division (*Tetramitus*); that, in somewhat

higher forms, its attractive force keeps the granules together during resting phases as well as during division (*Chilomonas*, *Trachelomonas*, etc.); and, finally, that in still higher forms a nuclear membrane is formed about the whole (*Euglena*, *Trachelomonads*, *Synura*, *ciliates*, etc.). But another line of development may have arisen from this primitive type. In some forms the archoplasm may have remained outside of the aggregate of chromatin granules, becoming secondarily centralized in the manner described by Schaudinn ('96) for the division of the flagellate-stage of *Paramoeba*, or in the same way as the central-spindle in *Noctiluca* becomes centralized. In this way the sphere in metazoan cells may have arisen without any connection with the intra-nuclear body of most Protozoa, while the centrosome, as Flemming ('97) suggests, is an organ of secondary importance in the cell and which may or may not be present.[1]

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[1] G. N. Calkins, The Phylogenetic Significance of Certain Protozoan Nuclei. *Ann. N. Y. Acad. Sci.*, vol. xi, no. 16, 1898.

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## DESCRIPTION OF PLATE XL.

Figs. 11, 16–19, 21, 22, 24, 26–31, 38 represent nuclei in the process of spore-formation; Figs. 1–10, 12–15, 20, 23, 25, 32–37, 39 are various stages of the vegetative nucleus. Picro-acetic was used for individuals represented in Figs. 1, 5, 8, 15, 19, 20, 24; sublimate-acetic (weaker solution) for Figs. 6, 7, 10, 13, 17, 18, 21–23, 25, 32–37, 39. Stronger solution for Figs. 26, 27, 29–31, 38; Hermann solution for Figs. 9, 11, 12, 14, 16, 28; and corrosive sublimate for Figs. 2, 3, 4. The iron haematoxylin was used in all cases save Fig. 10, where the Biondi-Ehrlich mixture was used. Figs. 1–9, 11–18, 20, 21 are from preparations *in toto;* Figs. 10, 19, 22–38, from sections. $x$ = supposed intra-nuclear centrosome.

Fig. 1. Resting stage of a vegetative nucleus showing 9 karyosomes and granular oxychromatin. Sphere on the outside of the nuclear membrane presents characteristic granular cortex and hyaline central part.

Fig. 2. Nucleus showing early stages in fragmentation of the karyosomes.

Fig. 3. Nucleus showing possible method of karyosome fragmentation. $x$ = the supposed intra-nuclear centrosome.

Fig. 4. Later stage in karyosome fragmentation. The granules are gathered in groups, each group representing a previous karyosome.

Fig. 5. A still later stage in karyosome fragmentation. The sphere appears homogeneous.

Fig. 6. A nucleus in the so-called "spireme-stage." The chromatin granules are still large, but are arranged in fibers. The supposed centrosome ($x$) is distinct from the chromatin.

Fig. 7. Same as Fig. 6.

Fig. 8. A nucleus in the prophase of division. The achromatin granules are arranged in fibers parallel with the chromosomes. The chromosomes are forming with the thickened ends towards the sphere. The sphere has become more dense, and the hyaline center is disappearing. Some of the karyosomes are not yet fragmented.

Fig. 9. A prophase of division. The granules are beginning to form fibers, —the chromosomes. The sphere is dividing. Numerous processes stretch out from the sphere into the cytoplasm. These are probably equivalent to astral fibers.

Fig. 10. The same stage in section. The nucleus is filled with granules which are forming chromosomes.

Fig. 11. Stage of the amphiaster. The chromosomes are all formed and are represented in optical section in the nuclear plate.

Fig. 12. An abnormal nucleus. The sphere is bent into an acute angle. The nucleus shows elongation in the primary axis before chromosome-formation.

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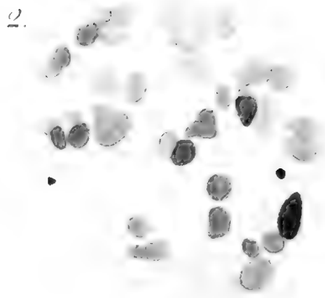

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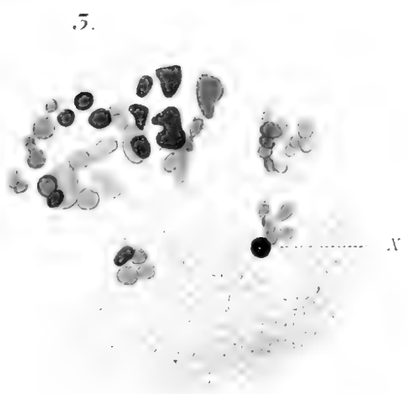


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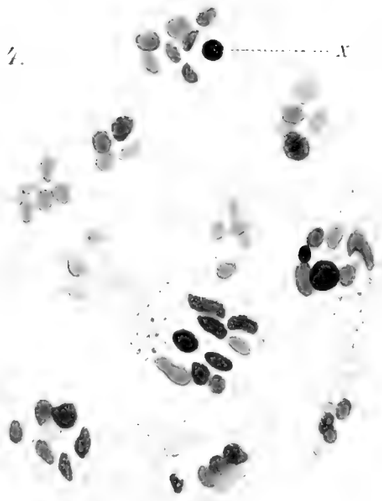


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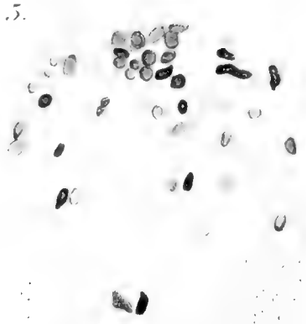

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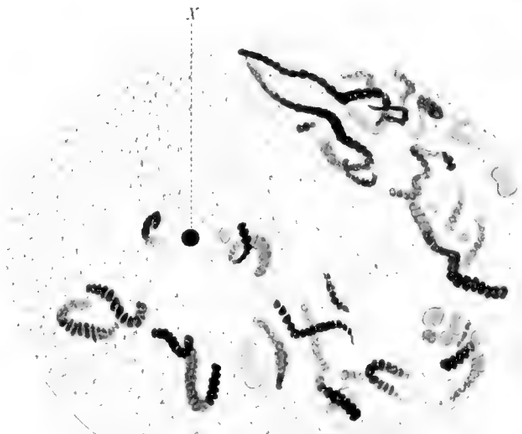


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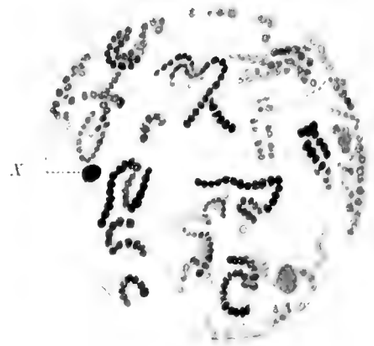


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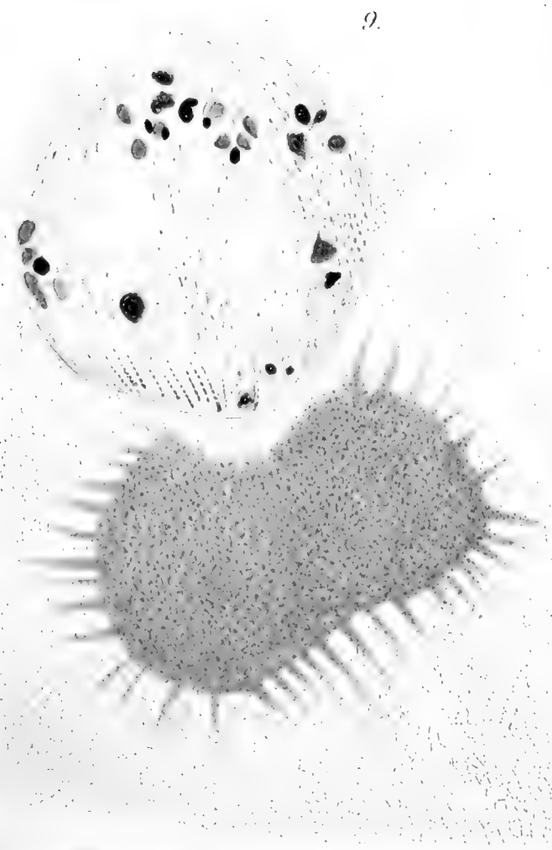

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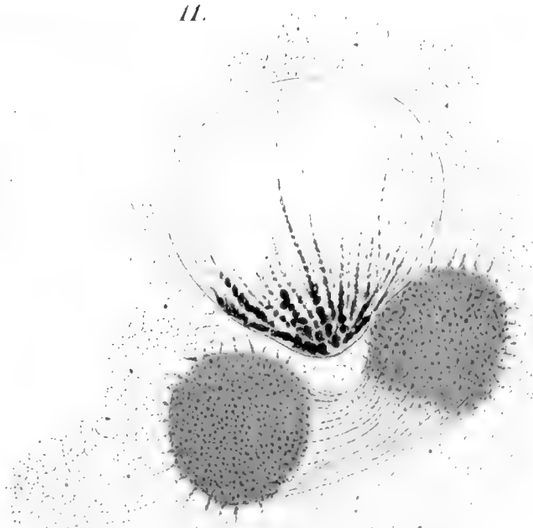

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## DESCRIPTION OF PLATE XLI.

FIG. 13. The nucleus and amphiaster during metaphase. The latter lies in the secondary axis. Central-spindle, nuclear plate, and asters are shown. Mantle-fibers not represented.

FIG. 14. Metaphase showing position of the nuclear plate and the dumb-bell-shaped amphiaster.

FIG. 15. Metaphase stage viewed from the opposite side, *i.e.*, the side away from the central-spindle. The chromosomes are seen in optical section.

FIG. 16. Late anaphase seen from side opposite the spindle. The chromosomes are completely separated and show secondary thickenings at the ends towards the spheres. The nucleus has elongated in the direction of the secondary axis.

FIG. 17. Late anaphase showing the position of the central-spindle; the chromosomes are completely separated.

FIG. 18. Telophase showing the formation of the secondary nuclear plates and just previous to the division of the daughter-spheres. The plane of the next division is therefore clearly indicated.

FIG. 19. Late anaphase showing final division of the chromosomes. The nucleus and the central-spindle were curved so that only one pole (the upper) was sectioned.

FIG. 20. Late anaphase in vegetative division. The chromatin of the chromosomes is reforming into large karyosomes. The daughter-spheres are beginning to expand and to lose their densely granular appearance, while the characteristic hyaline portion of the central part is plainly indicated, resembling the sphere in Fig. 1.

FIG. 21. Late telophase and beginning of the secondary divisions. The daughter-spheres have formed amphiasters, although the parent nucleus is not completely divided as shown by the large "Verbindungsstück."

FIG. 22. Section of nuclear-plate stage showing nuclear plate and fan-like arrangement of the chromosomes.

FIG. 23. Late anaphase showing the collection of chromatin into karyosomes. A centrosome (*C*) is plainly indicated at the focus of the mantle-fibers. There is no nuclear membrane between chromosomes and sphere.

FIG. 24. Similar anaphase showing aggregation of daughter-chromosomes as in Fig. 18. No karyosome-formation taking place. Centrosome and mantle-fibers present.

FIG. 25. Late telophase in vegetative division. Remnants of the daughter-chromosomes can still be made out, although most of the chromatin is now collected in a number of karyosomes.

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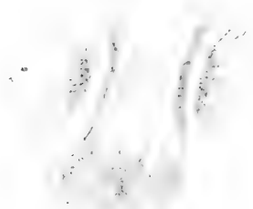

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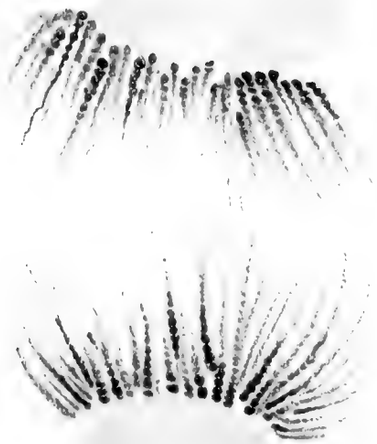

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## DESCRIPTION OF PLATE XLII.

FIG. 26. Transverse section showing the double nature of the chromosomes and compact arrangement of the proximal ends.

FIG. 27. Similar section showing one chromosome (*A*) beginning to split at the proximal end.

FIG. 28. Later stage. Mantle-fibers connect double centrosomes with the dividing chromosomes. Two separate bundles of daughter-chromosomes can already be made out.

FIG. 29. Division of chromosomes is carried still farther; the distal ends are not yet divided. In both figures the characteristic crossed appearance of this stage is plainly visible. The nuclear membrane persists except where mantle-fibers connect centrosome and chromosomes.

FIG. 30. A later stage in chromosome division. The distal ends are now separated. The nuclear membrane has reappeared between the central-spindle and the chromosomes.

FIG. 31. Horizontal section of similar stage. The central-spindle lies through the middle of the figure, the nuclear membrane is intact except at the poles. This is about the same stage represented in Fig. 17, and would represent a section cut in the plane of the paper.

FIG. 32. Prophase of vegetative division showing slight undulation in the membrane below the sphere, a hyaline space below the undulation, and two distinct granules in the hyaline space.

FIG. 33. Prophase of vegetative division showing very marked undulation of the membrane below the sphere, a distinct hyaline sphere, and again two granules within the space.

FIG. 34. Prophase of vegetative division. A distinct depression is now formed at the point where undulations appeared in the other cases. The two distinct granules are now seen in the space just outside of this depression.

FIG. 35. Prophase of vegetative division. The undulations are very marked in the region of the sphere, and are not found elsewhere. The two distinct granules are outside of the membrane.

FIG. 36. A similar stage. The two granules now lie in the sphere, the chromatin is in the so-called spireme-stage, and chromosomes are forming.

FIG. 37. The two granules are now very distinct and lie in the sphere. The nucleus is elongated in the primary axis, but the chromatin is still widely distributed.

FIG. 38. Section through primary axis of a daughter-nucleus in about the same stage as that shown in Fig. 18. The chromosomes are double, the mantle-fibers are distinctly granular, and the centrosome is double. The sphere has not yet divided.

FIG. 39. Late anaphase in vegetative division. The nuclear membrane is not yet reformed, the mantle-fibers are disappearing, and the chromosomes are disintegrating.

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G.W.C. del.

Litt. Anst. v. Werner & Winter, Frankfurt/M.


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