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J. S. KINGSLEY
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WITH THE COLLABORATION OF

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Columbia University

EDWIN G. CONKLIN
Princeton University

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Bussey Institution, Harvard University

WILLIAM PATTEN
Dartmouth College

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CONTENTS

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THE DEVELOPMENT OF PARAVORTEX GEMELLIPARA

(GRAFFILLA GEMELLIPARA LINTON)

STANLEY C. BALL

Department of Zoology, Yale University

SIXTEEN TEXT FIGURES AND NINE PLATES

CONTENTS

I. INTRODUCTION

1. Determination of the species

In the Brooks Memorial Volume of The Journal of Experimental Zoology, vol. 9, 1910, Prof. Edwin Linton described a new species of Rhabdocoele Turbellarian which he had discovered living in the ribbed mussel, Modiolus demissus. He referred it with some hesitation to the genus Graffilla (Jhering). Von Jhering ('80) in establishing the genus Graffilla characterized it in the following words:

> Pharynx nach Art des Mesostomeenschlundes aus Muskelfasern und eingelagerten Bindegewebszellen bestehend, nicht in einer Tasche gelegen, sondern direkt mit dem Munde zusammenhängend. Bursa copulatrix und Receptaculum seminis durch ein Organ vertreten. Ovarien langgestreckt, bandfömig, Dotterstöcke sehr reich verästelt.

Most important in the determination of the genus in which Linton's Turbellarian is to be placed is the question of the receptaculum seminis. Von Jhering in his figures of Graffilla muricicola indicates that two reservoirs are connected with the atrium (called by him the 'uterus'). The more anterior of these is the vesticulum seminalis which bears a short conical penis on its lower surface and projecting into the atrium. The receptaculum seminis lies just posterior to the seminal vesicle, and likewise dorsal to the atrium. It s to be noted that the latter is not merely a dorsal lobe of the atrium but is a distinct spherical reservoir, fully as large as the seminal

vesicle, and connected with the atrium by a slender duct of a length about equal to its own diameter. This canal as described by von Graff for Graffilla buccinicola is shorter but none the less definite. It is therefore characteristic of the genus Graffilla that the seminal receptacle is not sessile upon the atrium but connected with it by a canal. An examination of the Turbellarian discovered by Linton reveals no such reservoir.

This American species may be described as a Vorticid Rhabdocoele occasionally reaching 2 mm. in length and 0.80 mm. in diameter at its widest part; elongated ovoid in form while at rest, young specimens nearly linear when moving rapidly, posterior half of body somewhat broader, anterior quarter more flattened than central and posterior portion which is nearly cylindrical; ends bluntly pointed, the posterior often being the more acute; white with yellowish tinge, semi-transparent; body contractile and extremely flexible; crawls on bottom sometimes in a straight line, often performing circus movements; sometimes swims freely in water, revolving about its longitudinal axis and so follows a spiral course; pharynx sub-globose (doliiform) opening anteriorly into a small vestibule leading to the mouth; the latter is ventral but so close to the anterior end that it is directed forward; oesophagus not distinct from the spindle-shaped intestine into which it merges; intestine extends nearly to the posterior end, lies in upper half of body, walls thick, composed of large cells elongated toward the lumen; two black reniform eyes above the pharynx and anterior to the well developed two-lobed brain. Genital pore ventral at the end of the anterior third of the body, opening into a large atrium; prominent pear-shaped seminal vesicle carries at its lower end a muscular penis (without chitinous parts) which lies in a very small atrium masculinum; atrium produced posteriorly and dorsally into a pouch which acts as a bursa copulatrix; distensible antrum femininum extends posteriorly from the middle of the posterior wall of the atrium; antrum indistinctly forked at its posterior end to form two rudimentary oviducts, but usually so distended with spermatozoa (thus acting as bursa seminalis) that no forking is evident; two large horn-shaped ovaries one

on either side below the median frontal plane, extending from anterior fifth to middle of the body; two extensively branched and anastomosed vitellaria; ovaries and vitellaria not joined but closely apposed at their proximal ends where they meet the rudimentary oviducts; testes paired, situated one on each side just laterally and posteriorly to the pharynx; vasa deferentia short, arising from the inner posterior margins of the testes and opening into the seminal vesicle on its anterior surface; bursa seminalis lacking; viviparous, the young developing, usually in pairs, inside capsules of which as many as forty may be present at once in the mesenchyme of the mother; young break out of capsules and swim about in parent until they emerge through body wall or intestine; rolled up capsule shells remain in the 'mesenchyme.

Paul Hallez has given ('09) a detailed account of his observations on Paravortex cardii, a Rhabdocoele belonging to a genus closely related to Graffilla. The resemblance between Linton's and Hallez's species, as will be indicated below, is very striking.

The genus Paravortex was established by Wahl ('06) for a species of Turbellarian which is parasitic in the alimentary canal of Scrobicularia tenuis and S. piperata. According to Wahl it was discovered and inaccurately figured by Villot. Von Graff later called this parasite Macrostomum scrobicularіae, but Wahl asserted that it was worthy to be placed in a new genus. This he called Paravortex. He also found von Graff's Provortex tellinae to be identical with it.

Wahl ('06) briefly described the new genus Paravortex in the following words:

"Dalyelliide mit am Vorderende des Körpers gelegenem Pharynx, paarigen Keimstöcken, verzweiten Dotterstöcken, rundlichen Hoden und ventral vor der Körpermitte gelegener Geschlechtsöffnung."

In the same paper Wahl characterizes the genus Graffilla thus:

"Dalyelliide mit am Vorderende des Körpers gelegenem Pharynx, paarigen Keimstöcken von der Form gewunderen Bänder und davon getrenntren Dotterstöcken. Geschlechtsöffnung mittelständig, Hoden schlauchartig."

Concerning the presence or absence of a bursa seminalis Wahl says nothing, but his figures of the reproductive organs show very clearly that no such reservoir exists in Paravortex. We have already seen that this organ is characteristic of Graffilla.

According to Wahl, the genus Paravortex is distinguished from Graffilla, since its members have the genital pore situated in front of the middle of the body while in Graffilla this opening is at or behind the middle. The ovaries of Paravortex are club-shaped, while those of Graffilla are sinuous and band-shaped. The testes of Paravortex are nearly spherical; those of Graffilla appear bottle-shaped.

Von Graff gives ('08) a key to the Family Graffillidae, which is translated in part below:

A. With two germo-vitellaria and two compact, unlobed testes.
    1. Pharynx well developed, germo-vitellaria not lobed.....Vejdovskya
    2. Pharynx small, germo-vitellaria lobed in the form of a hand..Paravortex
B. With two ovaries and two vitellaria.
    1. Ovaries club-shaped, of normal size.
        a. Vitellaria long and unbranched.
            1. Testes paired, genital pore on ventral surface....Paravortex
            2 Testis single, genital pore at or near the posterior end .....Collastoma
        b. Vitellaria branched (Testes paired, genital pore near posterior end).....Umagilla
    2. Ovaries of different form, vitellaria branched or lobed.
        a. Ovaries abnormally long cylindrical cords, vitellaria lobed in form of a hand, genital pore central.....Graffilla
        b. Ovaries and vitellaria hand-shaped, genital pore at the posterior end.....Syndesmis

Thus it is seen that, according to von Graff, the genera Paravortex and Graffilla differ in that the former has two hand-shaped germo-vitellaria, i.e., the ovary and the branched vitellarium of each side unite at their proximal ends to form a single stalk which joins the oviduct. Graffilla, on the other hand, has two extremely long cylindrical ovaries which are not thus joined to the vitellaria; the latter, however, are branched.

Von Graff in his key did not refer to the receptaculum seminis, nor does he in the description of the genera dwell particularly upon this point. Nevertheless, while he fails to mention the organ in speaking of Paravortex, he notes that all the species

belonging to the genus Graffilla are furnished with "eine mächtige Bursa seminalis" (receptaculum seminis.)

In order to classify Paravortex cardii Hallez ('09) presents the following table which brings out more clearly, as I think, the generic differences between Paravortex and Graffilla.

1. A single ovary. Body more or less flattened.....(2)
   Ovaries and vitellaria paired.....(3)
2. Ovary large, irregularly lobed. Genital pore posterior.....Anaplodium
   Ovary small. Genital pore at the beginning of the last quarter of the body.....Didymorchis
3. Vitellaria unbranched.....Collastoma
   Vitellaria branched or reticulated.....(4)
4. Genital pore posterior.....(5)
   Genital pore ventral in the first half of the body.....(6)
5. Intestine lobed. A pharynx.....Syndesmis
   Intestine straight. No pharynx in adult.....Fecampia
6. A bursa seminalis.....Graffilla
   No bursa seminalis.....Paravortex

From Hallez's key it appears that there is a disagreement as to whether the ovaries and vitellaria in Paravortex should be considered as one organ. Von Graff asserts that they are united, while Hallez considers them separate. From his description of P. cardii it is evident that Hallez is correct.

In comparing the genera Paravortex and Graffilla Hallex says, "Les genres Graffilla and Paravortex ont entre eux des affinités très étroites. La seule différence vraimente importante qui existe entre ces deux genres c'est l'absence chez Paravortex de la bours séminale qui se recontre dans toutes les espèces de Graffilla."

From a consideration of the several descriptions and keys quoted above one may summarise the distinction between the two genera Graffilla and Paravortex in the following manner:

Graffilla	Paravortex
1. Two extremely long cylindrical ovaries.	1. Two club-shaped ovaries.
2 Genital pore ventral, not anterior to the middle of the body.	2. Genital pore ventral, anterior to the middle of the body.
3. A large and distinct bursa seminalis (receptaculum seminis).	3. Bursa seminalis lacking.
4. No vestibule between pharynx and mouth.	4. Vestibule.

It is therefore obvious, since it agrees in all four of these characteristics, that Linton's species must be referred to the genus Paravortex. It now remains to determine wherein, if at all, the American species differs from the two found in Europe. Of Paravortex cardii Hallez gave a detailed description which, translated, is as follows:

"Vorticid provided with two ovaries, two reticulated and anastomosing vitellogenous glands and two spherical testes; bursa seminalis lacking. Genital pore ventral, situated close behind the pharynx, at the end of the first quarter of the body. Male copulatory organ muscular, without chitinous parts and bearing two lobes furnished with papillae. Pharynx doliiform. Mouth ventral, near the anterior end of the body. Body cylindrical, thinner at the anterior end, uniformly ciliated, white and with a slight yellowish tinge, transparent, without rhabdites, very contractile, ordinarily bent in an arc and turning about in one spot so as to describe a circle. Two black reniform eyes above the pharynx. Maximum length, 1 mm.; diameter 0.3–0.4 mm. Viviparous. Numerous soft-shelled capsules (up to forty) each enclosing one to four embryos and distributed through the connective tissue. Empty and rolled-up shells remaining in the body of the mother. Lives in the stomach of Cardium edule."

Linton's species and P. cardii are closely similar both in structure and habits. Both have essentially the same color and the same shape of body. Both have similar digestive, sensory and glandular organs; both give birth to living young which develop in capsules within the mother's body; both show the same peculiar movements when taken from their host and placed in sea water.

The two species differ, however, in that the American form attains twice the size of P. cardii; the genital pore is situated farther posteriorly and the ovaries are longer in the latter; an atrial canal in P. cardii leads from the dorsal part of the atrium backward to the antrum femininum, while in the American species there is no distinct canal but rather the antrum femininum extends backward from the middle of the posterior

atrial surface and its opening into the latter is strongly constricted by a sphincter muscle; the openings of the shell glands in P. cardii are distributed along the entire ventral wall of the atrial canal and antrum femininum, while in Linton's species they all open at the anterior end of the antrum just back of the atrium; the vitello-oviducts of P. cardii are the longer. Linton's species lives as a commensal in the mantle cavity of the ribbed mussel, Modiolus demissus; P. cardii is parasitic in the stomach of Cardium edule.

Linton's species resembles Paravortex scrobicularia rather than P. cardii in the form of the ovaries, i.e., they are elongated in the first two and shorter in P. cardii. In form of antrum femininum and lack of an atrial canal Linton's species and P. scrobicularia are similar, but the latter, on the other hand, is similar to P. cardii rather than to the American form in that the atrium does not project dorsally beyond the opening of the antrum.

Since Linton's species obviously differs from the European forms, his specific name gemellipara is to be retained. Its applicability is owing to the fact that ninety-five per cent of the capsules contain two embryos.

2. Historical

While the anatomy and taxonomy of the Rhabdocoele Turbellaria have received considerable attention, only a few works have appeared dealing with their embryology. Hallez ('78) published the results of his observations upon the winter eggs of various Turbellaria. Since the young are enclosed within a hard-shelled capsule, the material was so difficult to study that he characterized these results as insufficient. In 1887 he did further work upon the fresh water Dendrocoeles.

In 1903 Caullery and Mesnil described the development of certain species of Fecampia, Rhabdocoeles parasitic in certain Crustacea.

One of the most complete and noteworthy contributions to the embryology of this group is that of Bresslau. In 1904 he published the results of his research, begun in 1898, upon the embryology of four species of the family Mesostomidae, namely

Mesostomum ehrenbergi, M. productum, M. lingua, Bothromesostomum personatum, and of one Alloiocoele, Plagiostomum girardi.

Another remarkable contribution to Rhabdocoele development is the section devoted to embryology by P. Hallez in his later work ('08), to which we have already referred, upon Paravortex cardii, a Rhabdocoele parasitic in the stomach and intestine of the mollusc Cardium edule. He therein reinterprets the significance of certain figures published by Caullery and Mesnil. As a result of the behavior of the ectoderm and entoderm cells to be described in this paper some of Hallez's figures in turn suggest an interpretation differing from his own. The condition at corresponding stages in the development of Paravortex cardii and P. gemellipara shows in many respects a striking similarity. In the formation of the ectoderm and the manner in which the vitellarial yolk enters the embryo, however, the process in P. gemellipara differs distinctly from that described for P. cardii. The ectoderm differentiation agrees essentially with that found by Bresslau in Mesostomum ehrenbergi. On the other hand, though the end result is the same, the manner in which the yolk is taken into the embryo of P. gemellipara presents, so far as the literature studied has revealed, marked variations from that described for any animal.

While Linton made no serious attempt to discover the origin of the twins in Paravortex gemellipara, he suggests that the twin condition might be due to a process of polyembryony. In order to settle this point J. T. Patterson in the summer of 1911 continued the study of the worm at the Woods Hole Laboratory. The results of his work appeared in 1912.

The purpose of the present paper is to describe the main features in the behavior and development of Paravortex gemellipara. Particular attention is to be paid to the processes of nutrition as taking place in all stages from the oocyte to the free-swimming worm, comparing them with homologous processes observed by investigators working on other forms.

It is a pleasure to express here my sincere appreciation of the kind assistance offered me in all stages of this research by Dr. Wesley R. Coe.

II. BIOLOGY, MATERIAL AND METHODS

Biology and material

Paravortex gemellipara was stated by Linton to be a commensal living on the gills of Modiolus demissus, but Patterson concluded that the worm lives primarily in the kidney, although he thinks it likely that many individuals escape and may then be found in the mantle cavity and on the gills. Patterson opened two dozen mussels, using care not to injure the tissues, When washed in water no parasites were found, but after tearing the kidneys of these same mussels apart, he secured thirty-eight specimens. Hallez ('09) found P. cardii in the stomach and intestine of Cardium edule. So, thinking that in attempting to dissect the kidney, Patterson might have cut the alimentary canal, I made several attempts between May and September to discover the worm in the stomach, intestine, kidney and liver.

After the adductor muscles alone had been carefully cut and the valves forced apart eight to sixteen mussels were vigorously washed in sea water. If more be opened at once the sediment renders the Turbellaria difficult to detect. The worms were allowed to settle to the bottom, and, the better to see the bottom of the dish, most of the water was slowly decanted. The vessel was then placed upon a dark surface in good light. In looking for the very young a hand lens was found useful. Next the stomachs of the same mussels were carefully slit open with dissecting scissors and the interior washed in another dish by means of jets from a pipette. Water was also forced through the intestine in the same manner. Finally, in a third vessel, the same mussels were again washed after tearing apart all the tissues, thus insuring the breaking open of the kidney, an organ extremely difficult to distinguish and dissect without disturbing other tissues.

It was found that two hundred thirty-seven mussels collected at various times during the year yielded with the first washing one hundred eleven specimens of Paravortex, with the second two and with the third six. In one instance thirty-seven worms

were removed from eight mussels after the adductors had been cut, and only a single one when the viscera had been dissected. Considering the numerous crevices between the gills, visceral mass and mantle it seems very improbable that those worms which were not dislodged during the first washing were within the alimentary canal or kidney, but became freed from the mantle cavity in the two subsequent rinsings. The evidence points to the latter as being the usual abode of Paravortex gemellipara.

The number of worms obtained from different lots of mussels varies immensely. Patterson is of the opinion that there is a periodicity in the reproductive activity of this animal, but it is probable that the difficulty which he sometimes found in obtaining specimens was merely due to the irregularity of their occurrence. It has often been observed that one pailful of mussels may yield an abundance of worms, while another collected in a few hours and from the same mussel bed may produce very few. The conclusion is that only a portion of the mussels in the bed contain commensals. The success in obtaining specimens, therefore, depends upon the selection of the proper mussels.

Although usually no accurate count of the number of worms taken from given numbers of molluscs has been kept, a great variation is known to occur in their distribution. In the vicinity of New Haven Paravortex is abundant in certain mussel beds and almost entirely wanting in others. Any situation between high and low tide marks seems to be as favorable as another. Not always do those mussels which receive the most sunlight, even in winter, yield the greatest number of worms. In December, beds which were exposed to bleak winds sometimes gave better results than others which were fastened to rocks in sheltered coves with southern exposure.

Although, doubtless on account of increased metabolic activity and hence more prolific reproduction, Paravortex is more abundant in warm weather, still specimens may be obtained without difficulty at all seasons. I have collected them during every month of the year. Hallez asserts that P. cardii is as abundant

in late autumn as in summer at Le Portel, nor does he find them less numerous in December and April. The greatest number of P. gemellipara obtained at New Haven was collected on November 9, 1912, when about 175 mussels yielded 310 worms. Only those easily detected with the naked eye were taken.

Linton states that he found this species negatively phototropic; that on leaving a dish containing specimens in strong light the worms were afterward found to have collected on the side opposite the source of light. My observations, on the other hand, point to a neutral reaction on the part of the older worms and a positive reaction of the younger. The adults are as often found on the light side of the dish as on the dark. Their observed tendency to secrete themselves beneath debris on the bottom of the dish was at first interpreted to signify that they were reacting negatively to light, but when it was ascertained that in a dish lacking such debris these older individuals seemed neutral it was concluded that thigmotropism was responsible for their hiding reactions. It has been noticed that an adult worm, upon coming in contact with a piece of mussel gill or other tissue, often crawls along in contact with it.

The young worms, on the other hand, are positively phototropic. If a number of them be placed in a watch glass and the latter then rapidly rotated so as to cause the collection of the worms at the center, as the water comes to rest they immediately swim vigorously toward the source of light. Only daylight was used. This experiment was made more striking by the introduction into the watch glass of several specimens of Aphanostoma. The latter, being negatively phototropic, as rapidly crawled to the darker side of the glass. Repeated trials were followed by the same reactions, the two groups of worms separating at the center and progressing in opposite directions.

There is a marked tendency on the part of the young worms to crawl up the side of the dish above the water line; death often resulted.

One can conceive that thigmotropism may be of value in causing the older worms to remain in the mussels. Linton

suggested that negative reaction to light brought about this result, but as explained above it is extremely unlikely that the adults are thus negatively reactive to daylight. The marked positive reaction of the young specimens is probably responsible to some extent for their leaving the host within which they develop and so gives them an opportunity to enter other mussels. In the fulfillment of this end the negative geotropism of these young worms may also be of service; it would tend to cause a migration upward toward the source of light.

When removed from their hosts the healthy worms of medium size glide along the bottom of the dish at the rate of about one millimeter per second. Linton noted that the course in one direction was not long held, but often changed from one side to the other following a turning movement of the anterior end of the animal. Frequently these older worms halt and turn rapidly about for several revolutions in a circle whose diameter does not exceed the length of the individual (fig. 2). Hallez noted this circus movement as being extremely frequent in the case of Paravortex cardii.

The young worms move with greater rapidity for their size and in a much more direct course than the older invididuals. Furthermore they follow one direction often for several inches, only deviating in order to avoid obstacles. As already stated this course is consistently toward the light source. Rarely does a young worm perform the circus movement.

It is interesting to note in passing that Paravortex gemellipara like other Turbellaria observed by von Graff, is itself subject to parasitism. Frequently while studying living worms under the microscope numbers of an hypotrichous Infusorian are seen moving over them. One worm was so completely covered with them that, when the cover slip was pressed, these Infusoria became separated in such a way as to give the impression that the epithelium of the worm was peeling off. The hypotrichs, being ciliated only on one side, and closely approximating the epithelial cells in size, appeared strikingly similar to them.

Methods

Observations made on the living worm slightly compressed beneath a cover slip enable one to make out the genital pore, ovaries, testes, seminal vesicle and vitellaria. But for the genital ducts this method proved unsatisfactory. In one instance a mass of spermatozoa was distinguished in the atrium of a specimen which lay with its ventral surface up.

The most reliable and complete data were obtained from sections of the worm. Of specimens killed in corrosive sublimate, Zenker's fluid and strong Flemming solution and subsequently stained with iron haematoxylin, those killed in Flemming solution gave the most satisfactory results, both as to preservation and stain. In most cases Orange G was used as a counter stain.

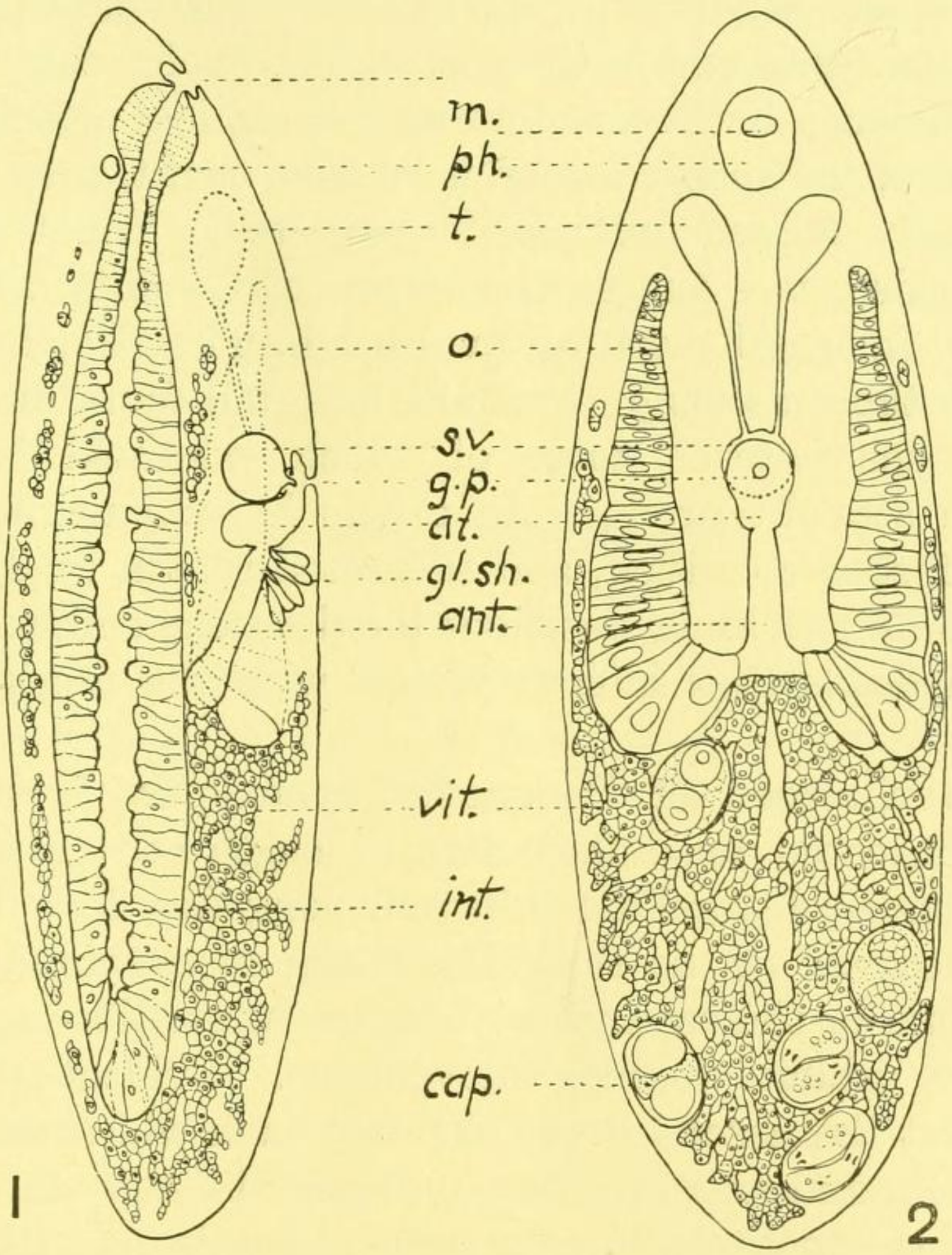
Material fixed in corrosive-acetic and stained in Ehrlich's haematoxylin was excellent for the study of tissues in which the exact condition of the chromatin was of minor importance. Nuclei, as a whole, show much more plainly when treated by this method than when iron haematoxylin is used. Here again orange G was used as a counter stain. In connection with the study of sectioned material living embryos of various stages, removed by pressure from the mother, furnished checks on many points. A few useful total mounts were prepared.

III. ANATOMY OF THE ADULT REPRODUCTIVE ORGANS

The male organs

Like the related forms described by Bresslau, Hallez, von Jhering and von Böhmig, Paravortex is protandrous. In half-grown individuals the male organs are well developed. The testes lie one on either side of the body close behind the pharynx (fig. 3). A large seminal vesicle lies just above the genital pore which opens in the midline of the ventral surface about one-third the distance from the anterior end of the body. Two short vasa deferentia lead from the inner posterior margins of the testes to the seminal vesicle. A common atrium intervenes between the seminal vesicle and ventral body wall in such a way that the plug-like penis, situated in the small atrium

masculinum at the lower end of the seminal vesicle, is directed through the common atrium toward the genital pore (text fig. 1). In no worm whose testes had become functional was the seminal vesicle found empty; usually it is distended with a mass of spermatozoa. All these organs are surrounded by a cellular wall.



Text fig. 1. Sagittal section of Paravortex gemellipara. × 167.

Text fig. 2 Optical frontal section from below. *ant.*, antrum; *at.*, atrium; *cap.*, capsules; *g.p.*, genital pore; *int.*, intestine; *m.*, mouth; *o*, ovary; *ph.*, pharynx; *s.v.*, seminal vesicle; *gl.sh.*, shell glands; *t*, testis; *vit.*, vitellarium. × 167.

In half-grown worms the male reproductive organs have reached maturity; after this period the testes with their ducts undergo partial degeneration. Patterson states that they are seldom found in large individuals, but I have seen them in every specimen examined.

Mitoses in the process of spermatogenesis are most readily observed in the younger worms; probably few spermatozoa are matured after the female organs begin to function. There is evidence, however, that this may happen. One series of sections of an old worm cut transversely shows a considerable number of apparently mature spermatozoa in the posterior dorsal portion of the testes and in the sperm ducts which can be traced for some distance backward toward the seminal vesicle.

The form of the spermatozoon is such as might, from its structure in nearly related animals, be expected. Von Graff ('02) figures and describes the spermatozoon of Graffilla buccinicola as 0.6 mm. in length with an oval head and a flagellum about twenty-five times as long. Von Jhering ('80) found in Graffilla muricicola spermatozoa some of which exhibited bifurcated heads with straight prongs. In others he was unable to observe this forked condition, and suggested that it might not be universal. Von Böhmig describes for the same species spermatozoa with heads thus bifurcated but with the prongs curved backward.

The spermatozoon of Paravortex gemellipara is composed of an unbifurcated head 0.01 mm. in length and an extremely slender cylindrical flagellum 0.10 mm. long (fig. 5). The head is slightly curved at the tip. The living spermatozoa are motionless while in the seminal vesicle, but when liberated in sea water they exhibit writhing movements. Evidently this behavior is not the normal mode of progression which takes place in the atrium and antrum, for no progress is made through the water; the spermatozoa merely twist about in one spot. Frequently they remain motionless in the water. Presumably the fluid inside the female passages ordinarily stimulates them to action.

The seminal vesicle in Paravortex gemellipara, as in the European species of Graffilla described by the German authors, functions not only as a reservoir but also as a 'ripening chamber.' Beside mature spermatozoa, many spermatids are usually found. Figure 48 was drawn from a living worm which had been compressed beneath a cover slip. In this seminal vesicle there were

about twenty-five spermatids at a stage so young that the differentiation of the flagellum had but just begun. The nuclei could readily be seen in those cells which lay near the surface. Numerous spermatozoa were present as well. On another occasion a living animal while under observation on a slide became crushed. Among the mesenchyme cells which escaped from the body were many spermatids. Each cell had the form of a thick concavo-convex lens attached to the flagellum at such an angle as to give the spermatid the appearance of a ladle. These spermatids are always present in the seminal vesicles of worms whose ovaries have begun to set eggs free, but in the very young in which the testes have just commenced to function all the sperm cells are mature on leaving the testis.

Female reproductive organs

As the male organs degenerate those of the female apparatus undergo rapid development. By the time the animal is two-thirds grown the ovaries and vitellaria are prominent. The ovaries are situated one on either side of the body below the median horizontal plane. At the small anterior end of each ovary, which lies just posterior and slightly dorsal to the testis of the same side, numerous oogonia are crowded irregularly together. Cell membranes can be distinguished except between the first two rows of nuclei. In some specimens a terminal filament passes forward from the tip of the ovary and joins an indistinct mass of cells on the posterior ventral surface of the testis. As will be seen in the part of this paper which treats the development of the reproductive glands, the ovary and testis arise from a common mass of undifferentiated cells, and later usually become separated. Where in later stages this strand of cells remains between the two glands it is regarded as a bridge which has failed to break down.

Striking spireme figures are to be observed in the oogonia. A short distance back from the anterior end of the ovary, however, only cells with resting nuclei occur. These are primary oocytes. Beyond this point the flattened cells are packed

closely together one behind another, as Patterson suggests, like a "rouleau of coins." The ovary increases greatly in diameter toward its posterior end. At about the mid-length of the animal it turns inward to meet the oviduct (figs. 3, 4; text fig. 2).

The enormous paired vitellaria discharge their contents into the same ducts as the ovaries. Each vitellarium sends branches anteriorly and posteriorly. At the height of their development the numerous anastomosed branches completely surround the intestine, occupying practically the whole region between it and the body wall (fig. 3). The anterior lobes lie above the ovaries and intestine; in extreme cases a few branches grow forward to a point above the brain. Single rows of yolk cells constitute the ultimate lobes. Indeed, individual cells frequently lie isolated in the parenchyma.

So closely do the vitellaria press upon the ovaries at the posterior ends of the latter and for some distance along their dorsal surfaces that the two organs occasionally appear to be joined into a single germo-vitellarium on each side of the body. As a matter of fact, however, if they join at all it is only at the point where they meet the oviduct—or better, the 'vitello-oviduct.' The stalks of the two vitellaria, as seen in figure 4, are separated only by a thin partition of mesenchyme. No cellular wall has been observed between the ends of the oviducts and the vitellaria. As shown in this figure, yolk cells often appear in the upper end of the antrum, indicating that they are likely to be liberated by any slight pressure in this region.

Posterior to the seminal vesicle a dorsal diverticulum of the atrium commune is considered by Patterson to be the degenerate seminal receptacle, although he found no spermatozoa in the sac. In several of our preparations it contains a mass of spermatozoa (fig. 9) which so distend it that the top is on a level with that of the seminal vesicle. This organ is rather to be regarded as a bursa copulatrix than as a receptaculum seminis; the spermatozoa, during copulation, are first received in this dorsal pouch of the atrium commune and afterwards pass into the antrum femininum. The atrium is lined internally with a high columnar epithelium, externally by a single layer

of flat cells (fig. 51). A comparison of text figure 1 with Hallez's brings out the difference between the two species as regards the shape of the atrium, atrial canal and antrum femininum. In P. cardii the atrium rises only a very little above the opening into the atrial canal, while in P. gemellipara the antrum opens into the atrium at the middle of its posterior surface. It was stated in the introduction that the lack of a bursa seminalis distinguishes the genus Paravortex from Graffilla.

The delicate-walled antrum femininum (text figs. 1, 2) extends from the atrium commune posteriorly and dorsally to a point beneath the intestine. Strong sphincter muscle fibers constrict the opening between antrum and atrium. So delicate are the walls that only occasionally can the cells be made out. Figure 4, referred to above, was drawn from a horizontally sectioned animal which showed the walls of the antrum exceptionally well. The section chosen included only the upper end of the antrum; the rest of the structure must be conceived as lying below the plane of the paper. No marked bifurcation is evident here on account of a distention with yolk cells; the ends of the vitellaria border the whole posterior surface of the antrum, while the terminal egg in each ovary presses inward against its outer angle. No uterus homologous to that found in many Turbellaria is present in Paravortex. In this regard the following extract from von Böhmig's contribution to Bronn's Klassen und Ordnungen des Thier-Reichs is of interest:

"Bei allen diesen, dient, wie u. a. schon Böhmig für Graffilla muricicola und Vedjovsky für Phaenocora hervorgehoben haben, das Atrium commune oder Antrum femininum als Uterus . . . zugeschrieben werden."

Beneath the antrum and posterior to it are groups of single-celled shell glands. Each contains a prominent nucleus and a considerable amount of granular cytoplasm (figs. 3, 9). Although it is difficult to determine the exact point at which these glands are attached to the reproductive tract, they all converge toward the lower end of the antrum near its union with the atrium. As noted above, this character helps to distinguish

P. gemellipara from P. cardii in which the shell glands open along the entire central surface of the antrum.

Although no one has recorded observations of Graffillidae in the act of copulation, it is inferred that pairing occurs. During the process, as described for other Turbellaria, masses of spermatozoa from the seminal vesicle of one animal are introduced by means of the penis into the atrium of its mate. There may be a mutual exchange, but certain observations lead to the belief that the younger specimens in which the male organs are at the height of their development fertilize the older ones having the functional female apparatus, while older ones do not fertilize the younger.

In this regard it is to be noted that during early life the animals have widely distended seminal vesicles; in the old worms this organ contains only small masses of spermatozoa. Moreover the younger specimens in which the ovaries are not functional have not been observed to contain sperm in the atrium and antrum.

Embryos

In a worm which is rapidly reproducing one finds distributed through the parenchyma capsules (fig. 1) containing embryos in various stages of development, from the newly-fertilized egg to ciliated young, actively moving about in their cramped quarters. During early development the two embryos of each capsule are usually separated by a considerable thickness of yolk, but may in some cases lie so close together that the blastomeres of one touch those of its mate. The capsular membrane, heavy and well defined while the embryos are small, becomes gradually thinner on account of water absorption and consequent stretching until, by the time the young develop cilia, it is extremely delicate and apt to be ruptured by vigorous pressure of the surrounding organs. When fully enough developed the young break forth from their capsules, swim freely about in the mesenchyme of the mother for a time, and ultimately, as her body undergoes degeneration, slip through the body-wall to the outside. Frequently one is found in the mother's intestine.

IV. DEVELOPMENT

1. Growth of the eggs and consideration of the yolk-nucleus (mitochondrial mass)

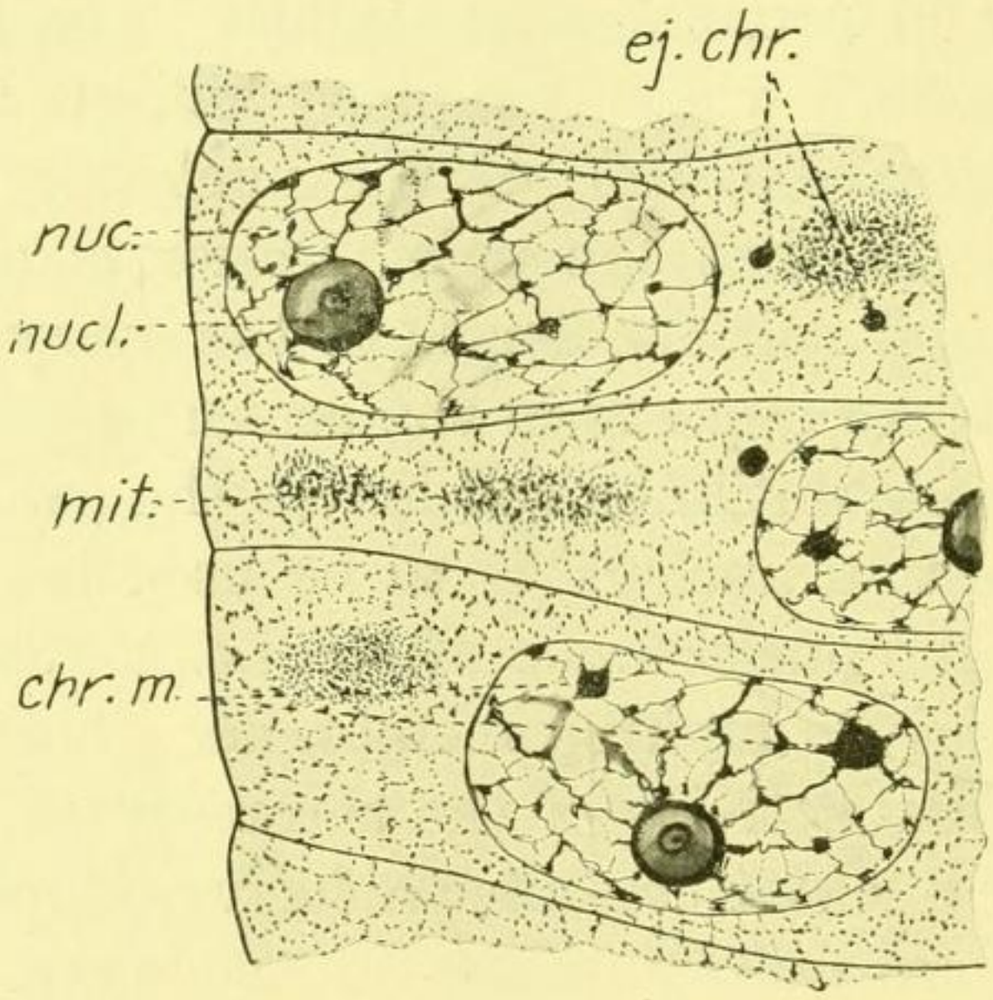
Throughout the entire length of the ovary (text fig. 2) from the point where the oogonia are transformed into oocytes there is a gradual increase in the size of the latter. The nucelus measures at the beginning 0.01 mm.; at the time it leaves the ovary its diameter is about 0.025 mm. During all this growth period the nucleus is in a resting condition, showing a fine chromatin reticulum and prominent nucleolus. The latter is at first apparently a sphere whose periphery retains more stain than the center. Often one side of the nucleolus is much darker than the other, so that the pale interior is eccentric. When the cell has passed half the length of the ovary a dark spherule with a light interior appears in the center of the nucleolus (figs. 7, 8), and remains until the cell leaves the ovary.

The term 'resting nucleus' is to be used here only in so far as it applies to the nucleus as a whole; i.e., it retains its spherical or oval form and its limiting membrane. There is observable, however, a certain activity of the chromatin. While the newly formed oocyte nucleus contains a heavy chromatin reticulum whose strands are quite uniform in thickness, the latter soon became markedly thinner. This is due to the accumulation of a part of the chromatin in one to four masses usually near the nuclear membrane (text fig. 3). These are at first irregular, but soon become rounded so as to resemble the nuleolus in form. Rarely do they attain the size of the latter, however, and never contain a lighter interior; an intense blue-black iron-haematoxylin stain is characteristic of these fragments.

It is first at this stage that one often finds one or more black bodies of perfectly spherical outline lying in the cytoplasm outside the nucleus. Their appearance is such as to suggest their identification with the chromatin bodies just described as arising in the chromatin reticulum. If now the nuclei of the cells in whose cytoplasm these black spheres lie be examined, it is found either that they contain only a nucleolus and a fine

chromatin reticulum, or that fewer black masses are present than in the oocytes which are slightly younger. It seems probable then that the oocyte nuclei eliminate a part of their chromatin in the form of spherical fragments which come to lie in the cytoplasm.

Of considerable interest in relation to the nourishment of the embryo is a body always found present in the oocyte after it has commenced its growth. This is the 'Dotterkern' or 'yolk-nucleus.' A light brown color in iron-haematoxylin-eosin



Text fig. 3 Portion of a frontal section of the ovary to show masses of chromatin before and after ejection from the nucleus. *chr.m.*, chromatin mass; *ej.chr.*, ejected chromatin; *nuc.*, nucleus; *nucl.*, nucleolus; *mit.*, mitochondrial mass. × 1016.

preparations brings the yolk-nucleus into contrast with the cytoplasm in which it is imbedded (figs. 4, 6, 7). At a magnification of two hundred fifty its appearance is that of a homogeneous gelatinous mass, sometimes existing as a single compact body, but often as flocculent material in one or more smaller masses of different sizes. The substance consists ultimately (fig. 7) of a multitude of granules which are so extremely fine and so closely grouped that a high magnification is necessary in order to distinguish them as such.

Since the term 'yolk-nucleus' has been applied by different authors to several different cell structures in many groups of animals, it seems advisable to pause here, and briefly to describe these various types. The name 'Dotterkern' was first given by Carus in 1850 to a body which had been described by von Wittich as lying in the cytoplasm of immature spider eggs (Lycosa, Tegenaria and Thomisus). Since then observers have reported the presence of a yolk-nucleus in the eggs of echinoderms, molluscs, worms (Trematodes), crustaceans, myriapods, insects, fishes, amphibians, birds and mammals, including man.

As explained in Korschelt and Heider's Textbook of Invertebrate Embryology, the bodies described for the various animals as yolk-nuclei may be classified in three groups, (*a*) true 'Dotterkerne' which are supposedly concerned with the deposition of yolk in the egg, (*b*) bodies more or less similar to yolk-nuclei in appearance, and (*c*) structures which are to be identified as attraction spheres.

Calkins in his preliminary notice published in 1895 on the origin and changes of the yolk-nucleus in the eggs of the earthworm, Lumbricus terrestris, points out that "the work of various observers may be divided into two classes according as the yolk-nucleus is conceived to be of cytoplasmic or nuclear origin."

As examples of the first class Calkins cited, among others, the work of Lubbock ('61) who regarded the yolk-nucleus as a thickening of the plasm forming the vitellus, of Sabatier('83) who asserted that in the spider the yolk-nucleus arises in the cytoplasm near the nucleus and wanders to the periphery where it degenerates; of Hall ('90) whose observations on the chick led him to the conclusion that the yolk-nucleus has its origin in a mass of granules near the germinal vesicle to which it may send out prolongations; and the work of Monticelli ('92) who claimed that in the Trematodes there is no connection between germinal vesicle and yolk -nucleus, but that the latter arises as a protoplasmic differentiation of the cytoplasm. Jordan ('93) came to a similar conclusion in regard to the yolk-nucleus of the newt.

Of investigators who considered the yolk-nucleus to be derived from the nucleus Calkins mentioned Shäfer ('83) who observed in the fowl's egg that the yolk-nucleus was connected with the germinal vesicle by numerous fine striations; Balbiani ('83) who claimed that in the Geophilidae it originates by direct transformation of the parts of the nucleolus of the germinal vesicle. Leydig ('88) saw the nucleolus in Triton become amoeboid and wander out into the cytoplasm where it finally broke up into granules around the periphery of the yolk. Finally he cites Henneguy ('93) and Balbiani ('93)who both found that in various eggs the yolk-nucleus originates from the nucleolus.

As a result of Calkins' own observations ('95) on the eggs of Lumbricus he concluded that the yolk-nucleus is of nuclear origin. He found it lying on the margin of the nucleus in direct connection at several points with the chromatin; the nucleolus takes no part in its formation. After migrating into the cytoplasm he conceived the granules of which it is composed as separating and forming the yolk plates after undergoing a change in chemical composition.

Wilson ('96), in "The Cell in Development and Heredity" speaks broadly of the yolk-nucleus, conveying the impression that already the body was beginning to be looked upon as not having, perhaps, so much importance as a functional cell organ as had previously been attributed to it. He says in part:

> Calkins' observations taken in connection with those of Balbiani, Van Bambeke, and other earlier workers give, however, strong evidence, as I believe, that the 'yolk-nucleus' of Lumbricus is derived, if not from the nucleus, at any rate from a substance nearly related with chromatin, which is afterward converted into cytoplasmic substance. It is certain, in this case, that the appearance of the yolk-nucleus is coincident with a rapid growth of the cytoplasm; but we cannot suppose that the latter grows entirely at the expense of the yolk-nucleus. More probably the yolk-nucleus supplies certain materials necessary to constructive metabolism, and it is not impossible that these may be ferments. We may perhaps interpret in the same manner the elimination of separate nuclear elements, (i.e., not forming a definite yolk-nucleus) as described by Van Bambeke, Mertens, v. Erlanger and many earlier writers.

The meaning of the yolk-nuclei of purely cytoplasmc origin is very obscure, and we have at present really no ground for assigning to them any particular function. It can only be said that their appearance coincides in time approximately with the period of greater constructive activity in the cytoplasm but there is no evidence of their direct participation in the yolk-formation, and we do not know whether they are active constructive physiological centers, or merely stores of reserve substances or degeneration products.

Crampton in 1899 published an account of the 'yolk-matrix' of an Ascidian egg (Molgula). He first noted its appearance in the living primary oocyte as "one or more small masses of highly refractive granules" lying in the cytoplasm at one side of the nucleus. Later, the granules become more numerous and are closely packed together into a cap-shaped mass which partly encloses the nucleus. Various chemical tests led him to the belief "that the yolk-matrix is an albuminous body of the cytoplasm, but perfectly agrees with the albuminous constituents of the nucleus," and that on this ground the yolk-nucleus is either directly derived from the nucleus or is formed under its immediate influence.

After a time the granules constituting the yolk-matrix separate, according to Crampton's observation, and spread throughout the cytoplasm in whose interalveolar substance they came to lie. Finally the granules enlarge to form the deutoplasm-spheres.

In the light of more recent research it would appear that these yolk-nucleus granules are mitochondria, and that Crampton's second alternative as to their origin is the correct one i.e., that they are formed under the influence of the nucleus, rather than directly from nuclear material.

One of the most prominent types of yolk-nucleus was described in 1898 by van Bambeke for the oocyte of the spider, Pholcus phalangioides. First appearing as a small group of granules near the nucleus, it develops into a definitely outlined, more or less cresentic mass lying in a clear portion of the cytoplasm. Increasing immensely in size the body grows around the nucleus, sometimes adhering to it for a time, but finally reaching the height of its development in the form of a cup within whose

hollow the greater part of the cell body lies. In other words, the yolk-nucleus is peripheral. In some sections it appears as a crescent reaching more than half way round the cell; in other planes which are at nearly right angles to the last a ring-like band is the picture presented by the yolk-nucleus.

From this point on Van Bambeke noted a disintegration of the 'corps-vitellin.' The essential difference in the fate of the granules into which this body fragments from that ascribed to them by earlier investigators of other forms, lies in their transformation into fat globules which are subsequently 're-sorbés etassimilés par le protoplasme ovulaire." In view of this van Bambeke suggests that it is on account of the resorption that the protoplasm is able to elaborate the deutoplasmic elements. It will be remembered that Calkins and Crampton recognized a direct transformation of yolk-nucleus granules into yolk spheres.

According to von Adelung in the Zoologisches Centralblat Samassa and Groben described for the parthenogenetic egg of the Daphnid, Moina rectirostris, an intensely staining body of characteristic form, which they called the polar body. Weissman and Ishkawa, however, observed the formation in the living egg of the true polar body. Finally Lepechkine in 1900 determined that the structure observed by Samassa and Groben is a true yolk-nucleus, and characterized it as follows

> Er ist dem Ei von dessen Ablage an bis zum 32-zelligen Stadium eigentümlich, wird durch Kernfärbemittel tingiert, gehört von Anfang an einem bestimmten, am animalen Pol gelegenen Blastomer an, ist von bestimmter Gestalt, gleichmässiger Konsistenz und enthält Vakuolen, löst sich beim Zerfall gleichsam in feinste Körnchen auf, wobei diese, dem Kern sich nähernd, denselben umgeben; der Dotterkern steht in Verbindung mit dem Kerncentrosom, welches seinen Zerfall herbeiführt; nach dem Zerfall des Dotterkerns treten in dem entsprechenden Blastomer Fettröpfchen auf, welche auf lange hinaus einem Bestandteil dieses Blastomers sowie seiner Tochterzellen ausmachen; aus dem den Dotterkern enthaltenden Blastomer enstehen die Genitalzellen des Keims; endlich giebt der Dotterkern in den ersten Furchungsstadien ein Plasmaklümpchen ab, welches zwischen die Eioberfläche und die Dottermembran zu liegen kommt.
>
> Was die Bedeutung des Dotterkerns betrifft, so ist der Verf. (mit van Bambeke, Wilson und Häcker) der Ansicht, dass derselbe eine

Rolle im Stoffwechsel spielt, und die Entwickelung und das Wachstum entweder das ganzen Eis oder eines bestimmten Blastomers begünstig.

From the foregoing review it is evident, first, that several different cell structures have been designated as yolk-nuclei, and second, that diverse views have been held as to the origin, function, if any, and the fate of the 'Dotterkern.' In the majority of instances where the latter was traced its disappearance as a concrete cell organ occurred in the oocyte. A part of the yolk-nucleus observed by Balbiani in Tegenaria, however, retained its individuality throughout cleavage and finally became located among the yolk-spheres in the abdomen of the young spider.

Since Lepechkine's work of 1900 a vast amount of literature has appeared dealing with mitochondria. It is apparently to this class of elements that the true yolk-nuclei described by him and earlier workers belong. Therefore it seems justifiable to replace the name yolk-nucleus with one which is more descriptive of its true nature. We propose to use in this paper the term 'mitochondrial mass.'

The cytoplasm or ooplasm of the oogonia is, in well preserved preparations, so finely granular that it resembles an opaque fluid. With the iron-haematoxylin method a light bluish-gray color is characteristic. If the sections be washed too long in the iron alum after their removal from the stain, and subsequently counter stained with orange G, the latter imparts to the cytoplasm of the oogonia and oocytes a yellowish brown tint. In such mounts the mitochondrial mass, which also takes the orange stain, is detected with difficulty. Those preparations, however, which retain a sufficient amount of the blue haematoxylin show the mass even in its earliest stages. Figure 6 was drawn from such an ovary. A small group of brownish granules in the third oocyte which shows a membrane on the right hand side of the ovary is the first indication of the mitochondrial mass. A study of the nuclei in the younger cells does not lead to the conclusion that the granules are of nuclear origin. They are rather to be regarded as bodies which have arisen in the cytoplasm under the influence of nuclear energy.

The question as to whether any function is to be assigned to the mitochondrial mass in Paravortex can be more intelligently considered after the conditions under which it exists as long as it remains visible have been fully described.

At a time approximately coincident with the appearance of the mitochondrial mass the cytoplasm of the oocyte becomes less dense, while its general color changes from the bluish gray of the oogonium to a more neutral tint bordering on the brown. So far as can be observed with the highest magnification obtainable this change in color is due not to an intercalation of yellowish particles but to a change in the staining reaction of the cytoplasm as a whole. The simultaneous increase in the amount of the latter is not caused by deposition of distinct deutoplasm spheres. At any rate there is no addition of particles which stain differently from the cytoplasm. Neither is growth the result of vacuolization; the mature oocytes in well preserved material never contain vacuoles. The only change observable, in so far as the consistency of the cytoplasm is concerned, is a tendency of the minute granules to become grouped in slightly larger units of irregular form. The appearance presented to the eye is that of a multitude of small flakes and flocculent bars between which intervene irregular colorless regions which in size closely approach that of the stained particles (fig. 7). These spaces become somewhat more conspicuous as the oocyte reaches maturity. It has been impossible to detect in them the 'mitosomes' which Wilson and others have described as lying in the protoplasmic trabeculae between the alveoli of the echinoderm egg.

Although, as pointed out above, no conspicuous spheres are to be found in the cytoplasm of Paravortex, it is apparent that the particles which are added must be of the nature of deutoplasm. Their distribution is remarkably homogeneous; in other words, the eggs are homolecithal or alecithal.

Nourishment enters the ovarian cells by osmosis from the parenchyma surrounding them, not, as Patterson states, in the form of particles ingested from the vitellaria which at some points come into close proximity with the ovary. The vitelline

cells contain flakes and globules of a substance which, by the haematoxylin-eosin method, are stained an orange brown, and are in size and density much more conspicuous than the particles suspended in the cytoplasm of the oocyte.

During the growth of the latter the mitochondrial mass has assumed conspicuous proportions. This relative increase in bulk is well illustrated in figure 6, which indicates as well that the substance may be accumulated in a single mass, or irregularly distributed throughout the cytoplasm. One might easily imagine, on account of the uneven edges of the masses from which lines of granules are often seen extending out into the cytoplasm, that the mitochondria are being directly transformed into the deutoplasm particles. Van Bamkebe ('98) points out that it is well to guard against such a conclusion, since he finds in the oocytes of Pholcus that the 'yolk-nucleus' granules first become transformed to fatty drops which are reabsorbed by the protoplasm, and suggests that possibly the latter then manufactures from this nutritive material the true deutoplasm globules. For the present, judgment in this matter of the function of the mitochondrial mass is suspended. It is to be noted that the body increases consistently in bulk while the cell is still in the ovary.

At the time of encapsulation the oocyte has attained a size about four times that which characterized its earliest appearance. The average measurements are approximately 0.112 mm. in width and 0.023 mm. in length, the cells being strongly flattened perpendicularly to the long axis of the ovary.

2. Formation of the capsule and origin of the twins

As to the manner in which the older eggs are nourished, Patterson asserts, "that the ova are at their upper margins absorbing yolk from the glands" (the vitellaria). This altogether erroneous interpretation will be considered below with another concerning the origin of the twins. Again Patterson states that,

> In consequence of this rapid growth certain retrogressive changes involving the cell membranes separating contiguous ova frequently

make their appearance. As a result two or even more nuclei may come to lie within one common yolk mass, which occupies the extreme tip of the ovary. In other words, a syncytium is formed here. In the vast majority of cases only two ova are involved so that the usual picture displayed in this region represents a binuclated yolk mass.

Patterson was evidently misled, both as to the relation of the ova to the cells of the vitellaria, and their relation to each other, by the phenomenon of oblique sections across thin membranes. Hardly a better example of this condition could be found than a longitudinal section of the Paravortex ovary, particularly of its posterior end where it turns inward to meet the oviduct. At this angle several of the ova are sure to be cut with varying degrees of obliquity, rendering the egg membranes correspondingly indistinct. The membrane between the last two ova included is the most likely to be cut at the sharpest angle, and hence the most difficult to observe.

Since all the eggs in the ovary are flattened nearly perpendicularly to the horizontal plane, a frontal section of the organ is all that is necessary to prove that very distinct membranes do exist between all ova, particularly at the posterior end of the ovary (fig. 8). A vertical section cut obliquely through the posterior angle of the ovary brings out the same fact.

Patterson's figure, introduced as evidence in favor of his syncytium theory, indicates very clearly to us that the ovary curved inward at the right. The egg having the larger nucleus was pressed into its neighbor containing the smaller nucleus. Since the plane of the section was more tangential to the first than to the second the former appears to be contained within the boundaries of the latter. The membrane between the two appears indistinct, not because it is undergoing degeneration, as Patterson would interpret the condition, but on account of the obliquity of the plane at which it is cut. Many of my preparations present an identical appearance.

In a similar manner I would account for the faintness of the boundaries between ova and vitelline cells in the same figure. Sections through the posterior end of the ovary and the overlying vitellaria are not usually perpendicular at all points to

the membranes of the eggs and of the vitelline cells, owing to irregularities in the contour of the ovary. Wherever the section is not thus perpendicular it is oblique, and hence the membrane is inconspicuous.

The masses of granules which Patterson interprets as streams of yolk entering the ova can be found in eggs which are not in contact with the vitellaria. In my own preparations I have considered these darker lines of granules as portions of the cytoplasm which retained more stain than that the rest of the cell.

It is evident, then, that Patterson was misled, both in regard to the relation of the contiguous ova in the posterior end of the ovary, and the relation of the ova to the vitellaria. Therefore I cannot agree with him, either that the vitelline material is pumped directly into the ova, nor that the breaking down of a membrane between two ova is part of a process by which two or more nuclei are enclosed in one capsule. No syncytium is found here. I am not content with the qualification which Patterson introduces as follows:

"However not in all cases do the two contiguous ova lose their intervening membranes, but some become completely surrounded by vitelline cells, which through a process of disintegration, form the yolk mass of the definitive capsule." The egg membranes are never so lost. It can be shown that in order reasonably to account for the multiplicity of embryos in capsules of Paravortex gemellipara it is unnecessary to resort to such an extraordinary mechanism as a syncytium in the ovary.

As to the manner in which two embryos come to lie in one capsule two possible hypotheses remain; first, that polyembryony occurs in P. gemellipara, or second, that two or more ova are liberated, and, together with yolk cells from the vitellaria, become simultaneously enclosed within one capsular membrane. That the first hypothesis is false there is no room for doubt. Capsules have often been observed in which both ova are undergoing maturation, a state which precludes the possibility that a single fertilized egg was enclosed in a capsule and

subsequently divided into two cells, each capsule developing into an embryo.

There remains the hypothesis that any capsule which contains a certain number of embryos originally had deposited in it the same number of eggs. A priori it is to be expected that such a process occurs here, for both von Jhering ('80) and von Böhmig ('86) observed in the uteri of the oviparous parasites Graffilla tethydicola and G. muricicola respectively capsules containing usually two ova; Böhmig states that each ovary contributes one egg. The same mechanism is probably involved in the formation of the capsules of the oviparous specimens mentioned above and in the viviparous Paravortex gemillipara. In the former two species the capsules pass out of the animal through the atrium and genital pore, while in the case of P. gemellipara they never enter the atrium, but break out of the antrum into the mesenchyme.

Finally Hallez ('09) observed the formation of the capsule in Paravortex. To quote:

> Chez Paravortex cardii, comme dans l'espèce précédente, il n'existe ni utérus, ni cavité d'aucune sorte au point de jonction du lécithogène, de l'ovaire et de l'oviducte.
>
> Quand le cocon commence à se former, il se produit un afflux de cellules vitelline autour des ovules mûrs qui ne sont pas encore complètement détachés de l'ovaire, afflux qui distend la membrane propre de l'ovaire et refoule le tissue conjonctif latéralment, c'est-à-dire du côté où la résistance est moindre. Il se forme ainsi une hernie qui s'accroît davantage à mesure que le nombre des cellules lécithofères augmente autour des ovules mûrs . . . .
>
> On observe à ce moment des spermatozoïdes dans la partie de l'oviducte voisine de l'ovaire . . . , tandisque l'atrium femelle et une partie de l'oviducte contient un cylindre d'une substance amorphe, transparente, coagulée par les réactifs . . . . Cette substance, qui ne peut être que la produit de sécrétion des glandes coquillières, adhère par places à la paroi de l'atrium comme si elle était glutineuse.

Hallez finds that from two to four ova are thus inclosed in a capsule. Since the ovaries of P. cardii consist at their posterior ends of several eggs, there is greater likelihood that more than one at a time will be freed than there is in the case of P. gemellipara. As a matter of fact Hallez finds a larger number

of capsules containing three or four embryos than is met with in the latter species.

a. The process in Paravortex gemellipara. The general plan and appearance of the ovaries, vitellaria and antrum of P. gemellipara supports the hypothesis that a similar process occurs here. As described in the introduction, the antrum femininum bifurcates at its distal end, sending a thin-walled branch, the oviduct, to meet the ends of the ovaries and vitellaria of each side.

Examination of the posterior end of either ovary in animals which have reached the egg-producing stage usually shows that the last, and hence the ripest, egg is being pressed inward toward the antrum by the ova behind it. Posteriorly the compact vitelline cells crowd the egg in a forward direction so that it appears, in a frontal section, as in figure 4. Here the terminal egg of each ovary is being subjected to the same conditions. The one on the right seems just in the act of being forced into the oviduct. Such is the usual picture, an egg from each ovary on the verge of entering the oviduct. Only a slightly greater pressure is required to complete the process. Suppose the worm were now to contract its body in this general region; the probable result would be that both ova would enter the antrum.

Most conclusive visual evidence is to be gained from a series of sections of a specimen which had been killed in the most critical stage, where two eggs had just been set free and were being surrounded with the yolk cells from the vitellaria. The sections are slightly oblique between a vertical longitudinal and a frontal plane, somewhat nearer the latter.

Figure 9 drawn from the specimen under consideration, shows what is taking place. Apparently the right ovary, part of which is included in the section, was distorted and crowded inward by the contraction of the animal in the fixing fluid. Otherwise it would have been impossible to have cut a section, which would include, as this does, seminal vesicle, antrum and ovary. Aside from the retention of a deeper stain these two ova are in the same condition as the eggs at the end of the ovary. The nuclei of both are resting, and their chromatin and nucleoli

are typical of ripe ova in which maturation has not yet begun. Owing to a relief of pressure both eggs are more nearly spherical than those in the ovary.

The yolk cells are arranged about these eggs in a loose envelope on all sides except that toward the ovary. Between the yolk cells and the eggs numerous spermatozoa appear. The latter can be traced throughout the length of the antrum and into the atrium where they form a compact mass.

This specimen leaves no doubt that two normal ova have been freed into a temporary cavity formed at the thin-walled distal end of the oviduct and partly bounded by the ovary, vitellarium and mesenchyme, and are being surrounded with a number of vitelline cells, between which numerous spermatozoa were actively moving at the moment of fixation. No proof is at hand that one of the eggs came from the right ovary and the other from the left, nor that both came from the right; either case is possible. Their presence near the end of one ovary may signify that both ova were derived from the latter. On the other hand, the possibility is not excluded that one egg came from the other ovary, and by pressure of the tissues was quickly shifted to its present position. In favor of this view it is to be observed in this series of sections that, while the right hand ovum is still in contact with the outer corner of the last egg in the ovary, its mate is entirely free, and has assumed a more nearly spherical shape—two characteristics which an egg would probably possess had it come from the other side of the body.

That one ovary may contribute two ova to the formation of some capsules, is clear when it is known that occasionally three embryos are found in a capsule. As stated in the introduction, two is the normal number, but Linton ('10) and Patterson ('12) both observed capsules with one and three embryos. My material shows further examples of both; and in addition one capsule containing four. Thus it is possible that the two eggs deposited in any capsule may have had a common source, or may have come from opposite sides of the body. The weight of the evidence favors the latter as being of most common occurrence in Paravortex gemellipara.

In figure 9 are shown several masses of a homogeneous substance which is altogether unlike anything observed in other preparations. This latter fact, together with the arrangement of the masses—more apparent in other sections of the same capsule—in a half circle about the yolk cells which surround the two ova, suggests that the substance is the fluid secretion from the shell glands in the process of being laid down in the formation of the capsular wall. A similar condition was observed by Hallez ('09) for Paravortex cardii.

As the above quotation from his paper points out, Hallez asserts that there is no uterus in Paravortex cardii nor a cavity of any sort at the point of junction between vitellarium, ovary and oviduct. Still he figures a direct continuation of the lumen of the oviduct into the cavity which is receiving a mass of yolk cells. It is true that P. gemellipara possesses no permanent, distinctly limited uterus by whose contractions the yolk cells and ova are molded into a spherical mass to which by accretion the capsular fluid is applied. Nevertheless it is apparent that in P. cardii and P. gemellipara the process is essentially the same as in oviparous worms, differing only because the encapsulation cavity is bounded partly by the ruptured and distended end of the antrum femininum and partly by the vitellaria, mesenchyme and ovary. The cavity is indefinite and probably never exactly the same for successive capsules, but there is, in spite of the delicateness of the tissues, sufficient resistance to round off the capsular contents and allow of the laying down of the shell. Thus the process is essentially in agreement with that found by other investigators in related species.

b. Description of the newly formed capsule. The appearance of a capsule which has just passed from the reproductive organs into the body parenchyma is clearly shown in figures 10 and 11. It lay close outside the uterus, slightly posterior and ventral to the point at which the ovary turned inward to join the vitellaria. It should first be made clear that, whereas living capsules due to their turgidity are smooth in outline, preserved material shows capsules somewhat shrunken and crowded out of shape by neighboring tissues.

The shell membrane is clearly visible, on one side closely opposed to the yolk, while on the other a shrinkage space intervenes. This capsule contained but one egg. That illustrated in text figure 4 shows the two ova at once. Only exceptionally do eggs lie so near the surface as indicated in figures 10 and 11; usually they are until late cleavage entirely surrounded by yolk. In figure 11 the plane passed through portions of eleven yolk cells, of which there were, as determined by a count of the visible nuclei, thirty-six originally enclosed in the capsule. Hallez found from ninety to one hundred eighty-seven in various capsules of P. cardii. As may be seen by referring to figure 10 of another section through the same capsule several cells have already lost their membranes; only the degenerating nuclei remain to indicate the number of cells.

c. Behavior of the vitelline cells. Up to this point, with the exception of the yolk nucleus above described, the structure and behavior of the reproductive organs and their products are remarkably similar in P. cardii and P. gemellipara. From here on, however, the latter presents characteristic differences as to the fate of the vitellarial yolk-cell nuclei, absorption of the yolk and the formation of the ectoderm.

The ectolecithal yolk cells in the P. cardii capsule, according to Hallez, soon lose their membranes, those near the center retaining them the longest. About one-half of their nuclei, it is to be noted, degenerate, while the remainder come to lie in the portion of the yolk which stains like cytoplasm. From this portion the 'ergatoplasmic' granules of yolk have previously separated and collected in 'balles vitellines.' This second form of yolk stains distinctly with eosin. The cytoplasmic-like yolk occupies the periphery of the capsule, sending a prominent lamella inward between the embryos—now in process of segmentation—and secondary "parois alvéolaires plus où moins complètes autour des masses éosinophiles." With its 'migratory' nuclei the cytoplasmic yolk stands as a syncytium surrounding embryos and 'eosinophile' yolk. Hallez is of the opinion that these migatory nuclei are effective in changing the nature of the

yolk in the 'balles vitellines.' To carry out this function they migrate inward from the periphery.

He finds that the cytoplasm surrounding one of these migratory nuclei makes its way into the substance of each vitelline sphere, eventually drawing with it the nucleus. Later many of these nuclei again emigrate and may then enter into the formation of the ectoderm or of the primary intestine.

Thus it is to be borne in mind that, if Hallez's interpretation be correct, both the entoderm and at least a part of the ectoderm cells in the definitive embryo contain nuclei and cytoplasm which have undergone little change since leaving the vitellaria; in other words, the relationship of these cells to those derived from the egg is apparent only when one recalls that ovary and vitellarium were at their beginning differentiated from the same mass of cells.

Bresslau ('04) traced the history of the yolk cells in Mesostomum ehrenbergi, M. productum, M. lingua, Bothromesostomum personatum and Plagiostomum girardi. The yolk cells of the former behave like those of P. cardii in that they soon separate into two groups. The outer ones become flattened so as to form a shell membrane between the capsular shell and the inner yolk cells. The latter become vacuolated to such a remarkable extent that the nucleus and a small amount of cytoplasm is finally flattened in the form of a thin cresent on one side of the vacuole As the ectoderm formed from the anterior portion of the developing embryo envelops these yolk cells, the nuclei of the latter gradually degenerate, but may still be discerned between the larger vacuoles after the ectoderm has closed over the posterior end of the embryo.

No such shell membrane is formed in the capsule of Bothromesostomum personatum; here the much more rapid degeneration of yolk nuclei is accompanied by the appearance of multitudes of vacuoles of various sizes, all much smaller than those described for M. ehrenbergi. The formation of the ectoderm and enclosure of the yolk globules within the embryo is essentially the same as in the latter species.

The development of M. lingua and M. productum is so similar that Bresslau figures only the former. While a limited number of yolk-cell nuclei come to lie next to the shell the others rapidly disintegrate. No distinct shell membrane is indicated

The newly formed capsules of the Alloiocoele, Plagiostomum girardi, are stated by Bresslau to contain from ten to twelve eggs distributed amongst several hundred yolk cells. The latter then arrange themselves in groups about the several eggs. In the behavior of the yolk-cell contents a notable difference is to be observed in comparing it with the process of yolk transformation in Mesosotomum. The vitelline substance consists of a large number of small refractive spherules which collect at the periphery of the cells. Gradually the cell membranes disintegrate, the process setting in first next to the egg. Since the nuclei, like those in M. ehrenbergi, remain distinct for some time a yolk syncytium results. Vacuolization does not occur until after the embryonic ectoderm has begun to grow out over the yolk. As in three of the four species of Mesostomum a small number of yolk-nuclei become flattened on the surface of the vitelline mass, while the remainder, although less conspicuous than at first, remain visible, scattered through its interior until the ectoderm has enveloped the yolk. Here again no shell membrane is developed form the yolk cells.

For the Dendrocoeles, Planaria torva, P. polychroa and Dendrocoelum lacteum, Mattieson ('04) found that about one hundred and fifty of the yolk cells immediately surrounding the egg become separated from those farther removed and pass over into a syncytium. This change takes place before the enclosed embryo has reached the two-celled stage. Mattieson concluded that, even previously to the formation of the syncytium, an active interchange of material between the egg and yolk cells takes place. The nuclei of the yolk cells which enter this syncytium slowly degenerate, but are still met with at the stage when the pharynx is differentiated.

Two ectoderms are developed in these Dendrocoeles. First a transitory epithelium is formed by blastomeres which migrate through the yolk and arrange themselves in an extremely thin

sheet over the surface of the yolk syncytium. Later the definitive ectoderm, arising also from derivatives of the blastomeres, is laid down beneath the first.

To summarize: Bresslau ('04) observed the development of a distinct shell membrane about the yolk mass in the capsule of Mesostomum ehrenbergi. It was derived by a differentiation of the outermost vitellarial cells. In Mesostomum lingua and Plagiostomum girardi he found occasional nuclei lying in small masses of cytoplasm at the periphery of the yolk spheres, while in the case of Bothromesostomum all of the yolk nuclei degenerated; none showed any tendency to form a membrane at the surface of the vitelline mass. On the other hand Mattieson ('04) determined that a 'primary ectoderm' was established about the yolk-syncytim of Planaria torva, P. polychroa and Dendrocoelum lacteum by the union of migatory blastomeres.

According to Hallez's interpretation, certain of the yolk-cell nuclei in Paravortex cardii not only form a peripheral syncytium outside the embryos and the nutritive portion of the yolk, but later enter into the formation of the permanent ectoderm as well as the primary intestine.

The behavior of the yolk cells enclosed in the capsule of P. gemellipara resembles that in the case of Bothromesostomum personatum in that all their membranes immediately disintegrate. The process is so rapid that in two hundred and seventeen worms sectioned only one capsule (fig. 11) was encounted where a considerable part of these cell membranes remained intact. As in Bothromesostomum also, the yolk-cell nuclei at once begin to degenerate. Ordinarily they have disappeared altogether by the time cleavage is well under way, but occasionally degenerate masses of chromatin embedded in small islands of nucleoplasm remain visible until the primary entoderm cells have been differentiated. No membrane or temporary ectoderm is formed by the peripheral yolk cells.

The yolk in perserved material, whether enclosed within a cell membrane or lying free in the capsule, consists of multitudes of small globules and flakes lying in a clear plasma. On the whole, the appearance of the yolk in a newly-formed capsule

is very similar to that in the vitellaria. Since in living yolk all particles exist as either spherical, oval or oblong granules (figs. 12, 15) it is probable that the flakes present in preserved material are globules exploded by the action of reagents.

The nuclei of the cells shown in figure 11, whose membranes still remain intact, are already beginning to disintegrate. Succeeding stages of degenerating yolk-cell nuclei appear throughout this section as well as that represented in figure 10.

Occasionally individual yolk cells, perhaps endowed with unusual vitality, retain the membrane until late cleavage in the condition presented when the cell was enclosed in the capsule.

3. Maturation and fertilization

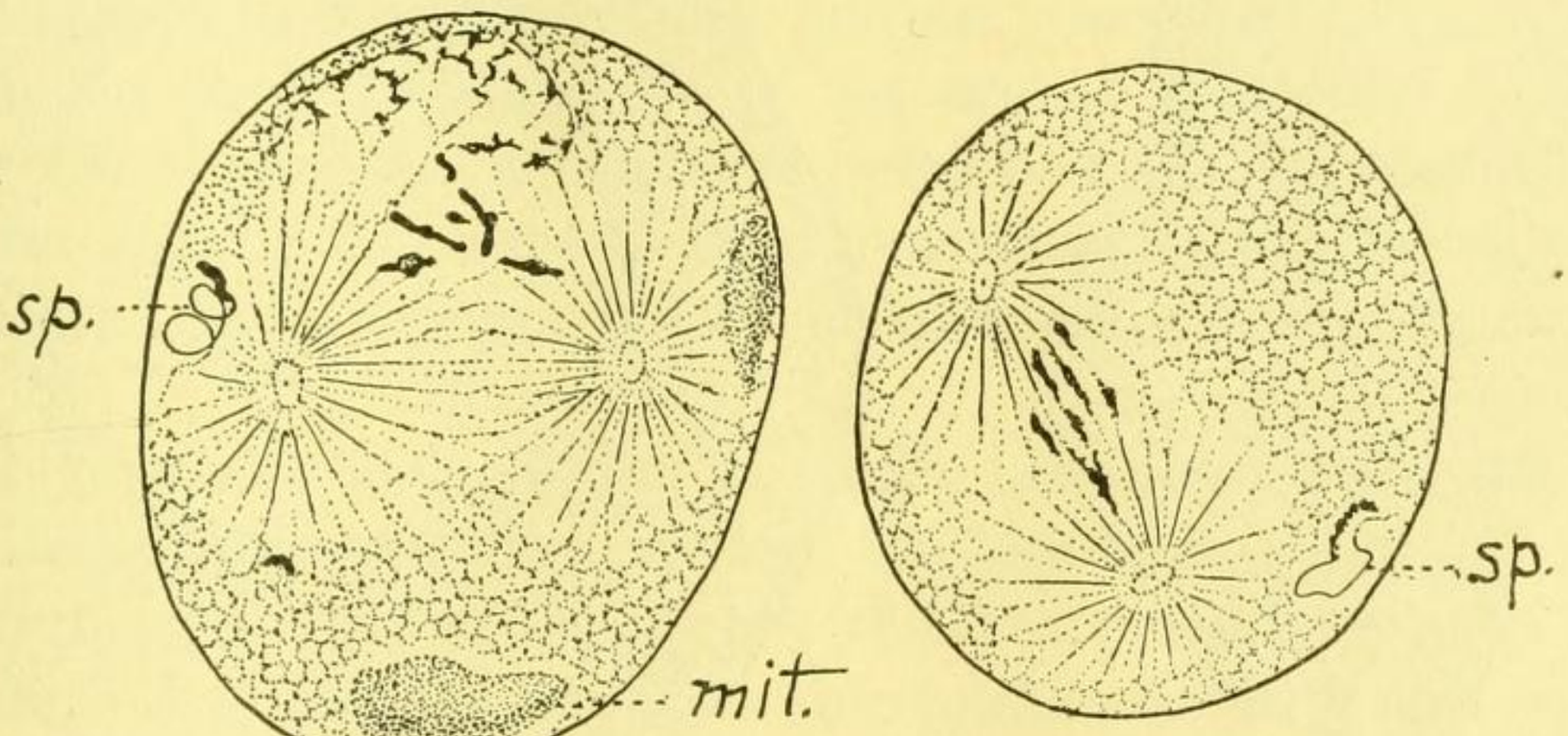
a. Insemination. That the egg in P. gemellipara may be entered by the spermatozoon immediately after leaving the ovary is plainly indicated in figure 9. which shows several spermatozoa between the two eggs and surrounding yolk cells. A careful search through all the sections of these ova yields no evidence that either one has been penetrated.

Each egg of another capsule, illustrated by text figure 4, about which the shell membrane had been formed, contains a spermatozoon. Either insemination has just taken place or the spermatozoon remains quiescent for some time before forming the male pronucleus, for both of these show plainly the long, deeply-stained head and a delicate coiled flagellum.

Without doubt many spermatozoa become enclosed in each capsule, where, as Patterson points out, their identity becomes obscured by the yolk granules. Occasionally a mass of sperm occupies a vacuole in the yolk; such a condition is shown in text figure 5. Presumably the sperm cells continue active in the capsule for some time, so that any egg that is not inseminated at the time it enters the capsule may later encounter the sperm.

Several other eggs have been observed in which the male pronucleus had become lobulated or amoeboid in shape, and had approached, in some a lesser and in others a greater distance toward the female element (text fig. 7).

b. Maturation. Patterson described an achromatic figure as appearing in the egg before it leaves the ovary, and remaining until after the formation of the capsule. In his paper ('12) on Graffilla (Paravortex) gemellipara he gave some space to an account of this spindle, noting the remarkable characteristics of its behavior. Although he believed at that time that the spindle later disappeared, he came to the conclusion after a study of the uterine spindle of Planocera that it was not entirely eliminated, but simply contracted to so small a size as to escape easy detection, and was only an early condition of the maturation spindle.

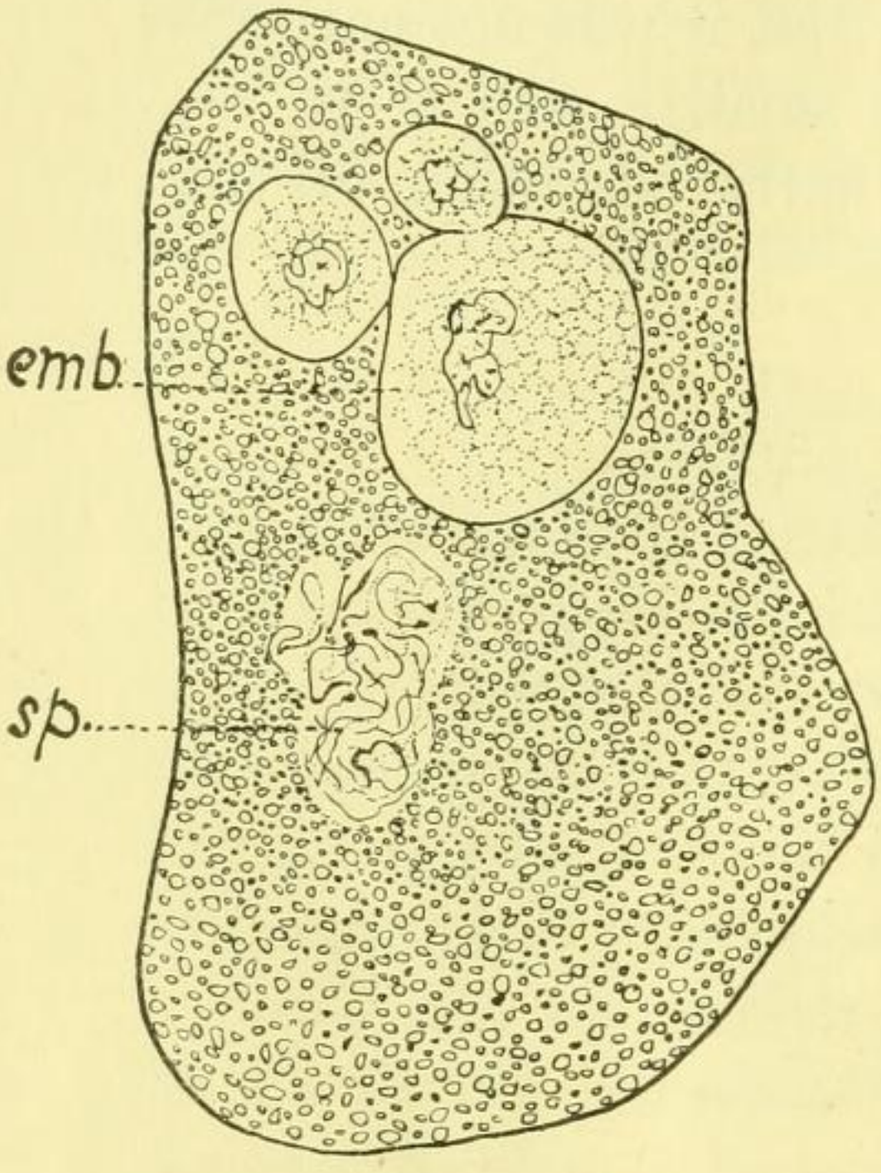


Text fig. 4 Section through two recently fertilized eggs of one capsule. Yolk and capsular membrane not shown. *mit.*, mitochondrial mass; *sp.*, spermatozoon. × 730.

Although occasionally a faint suggestion of a radial arrangement of the granules is perceptible in the yolk-nucleus mentioned above, in all my material no egg shows a true spindle until it has left the ovary and has become surrounded with yolk cells in the distal end of the antrum. It has, of course, for sometime been accessible to spermatozoa. A single egg was observed thus enclosed in loosely massed vitelline cells. It contained a large spindle occupying nearly the entire diameter of the egg. An exact count of the chromosomes in this spindle is impossible on account of poor preservation, but certainly more than the reduced number, four, are present This fact,

with the absence of any polar body, stamps it as the first polar spindle. Its appearance thus before the egg has been enclosed in its capsule is believed to be unusual.

The first maturation spindle typically appears after the two ova and their yolk cells have been encapsulated. At the time of its greatest length the spindle occupies nearly the entire polar



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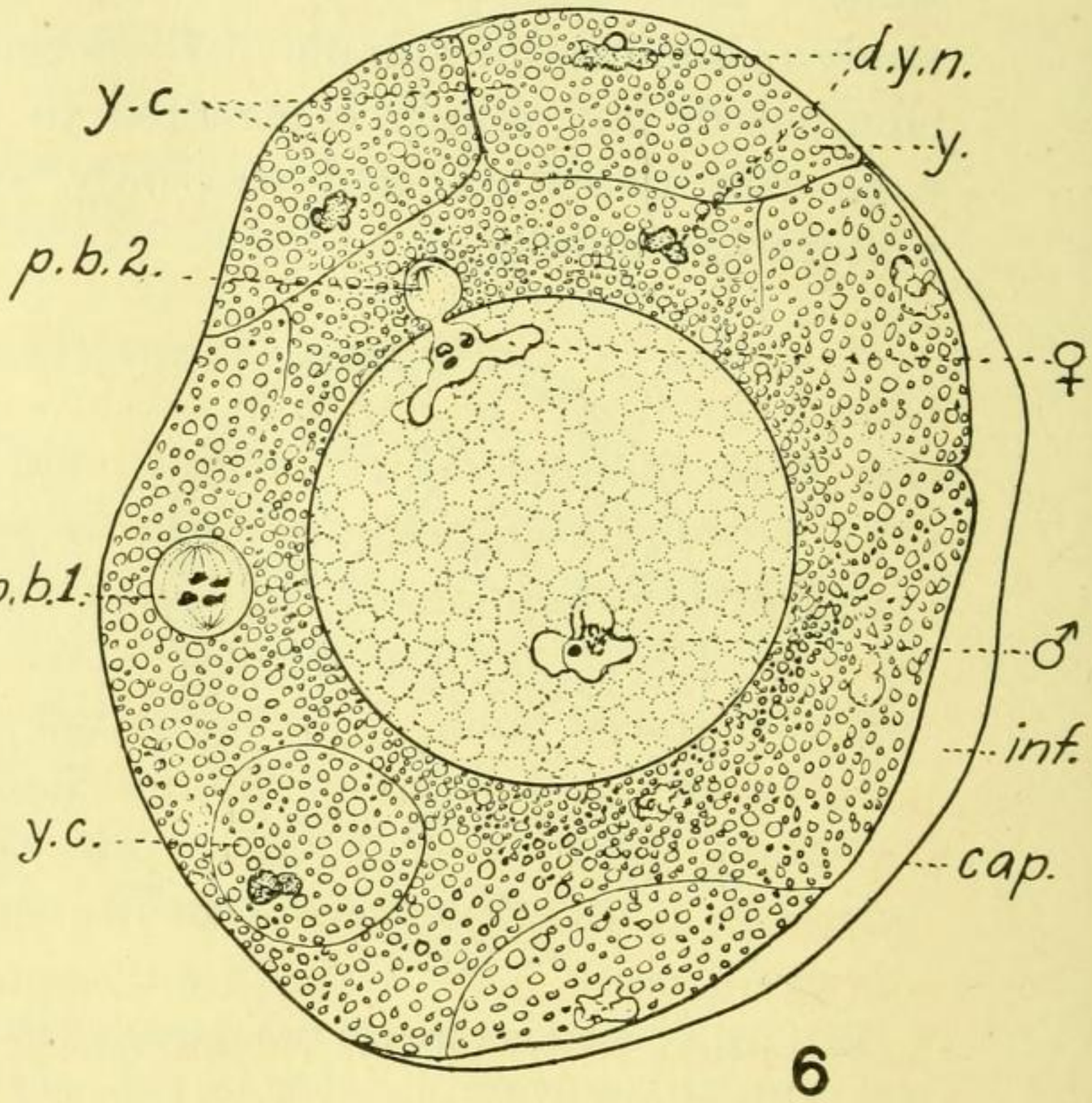

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Text fig. 5 Section of a capsule containing in the yolk a vacuole filled with spermatozoa. *emb.*, embryo; *sp.*, spermatozoa. × 400.

Text fig. 6 Reconstructed section in a plane perpendicular to actual sections through a capsule containing an egg which has given off both polar bodies. *cap.*, capsule; *d.y.n.*, degenerating yolk cell nuclei; *inf.*, infiltration; *p.b.1*, *p.b.2*, first and second polar bodies; *y*, yolk; y.c., yolk cells. × 730.

diameter of the egg (text fig. 8) Each centriole appears as a tiny dot in the center of a large centrosphere, from which radiate the conspicuous astral rays. Owing to the minuteness of the chromosomes, and the extreme difficulty in obtaining all the stages of the process of maturation, it is hard to determine in any given case just what phase they represent. One must depend rather on the presence or absence of polar bodies and

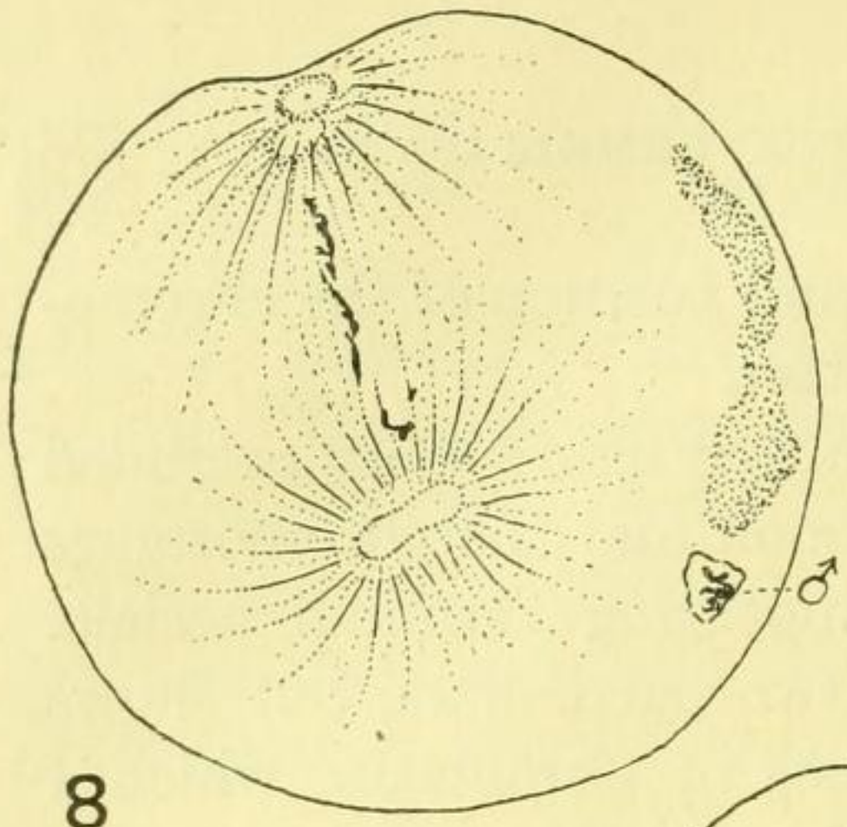
spermatozoon, and upon the number and position of the chromosomes, than upon the shape of the latter.

The egg at the left in the capsule in text figure 4 has extruded no polar bodies, contains a spermatozoon and the chromosomes are just being drawn from the disappearing germinal vesicle. Its mate agrees with it in the first two particulars, but shows an advance in that eight small masses of chromatin, which I conclude to be diads, are present in the spindle; in other words, the cell is in the early metaphase. Text figure 8 pictures a similar condition, except that it is impossible to distinguish all the chromosomes. One centrosphere has already divided, while the other is in the process of division.

Hallez ('09) determined that sixteen 'caryomerites' are present in the first polar spindle of the egg of P. cardi. Eight of these are eliminated in the first polar body. The second maturation spindle contains, as he expresses it, "four chromosomes (= eight caryomerites), half of which enter the second polar body."

My material yielded but a single instance where the first polar body had been given off, and the second maturation spindle was forming. In this egg (fig. 9) the first polar body, not quite detached, has already begun to degenerate, as indicated by the scattered chromatin. The centrosome remaining in the egg has divided to form the second polar spindle whose fibers are drawing the four diads toward the equatorial plate. Another section contains the spermatozoon which has begun to enlarge to form the male pronucleus.

In the preparation which best shows the first and second polar bodies the capsule was sectioned in such a plane that portions of the second polar body, though still connected with the egg by a protoplasmic bridge, lie within two of the serial sections, neither of which passes through the egg itself. A third section, however, contains a thin tangential portion of the egg showing the female pronucleus with small masses of chromatin. Text figure 6 represents a reconstruction of this capsule. The female pronucleus is lying just inside the vitelline membrane in the animal pole of the egg. Lobule formation has again set in.



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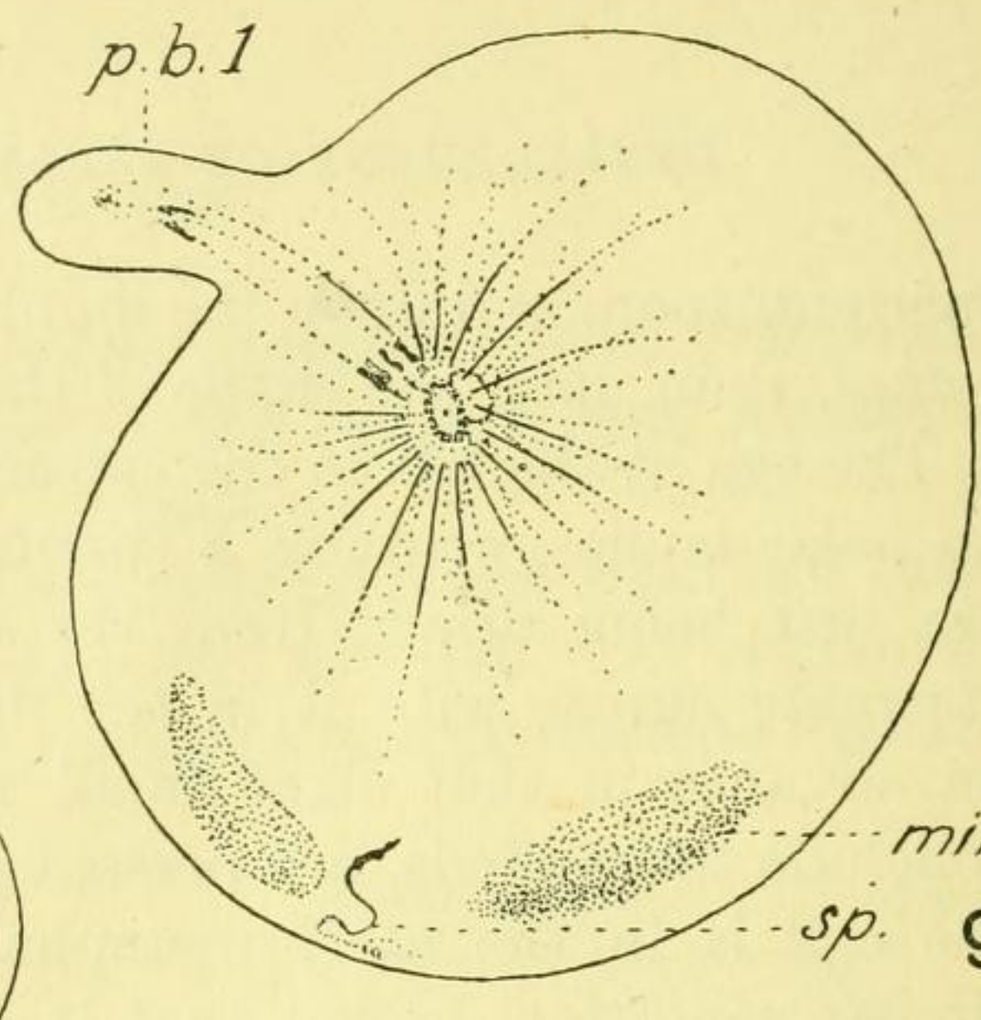
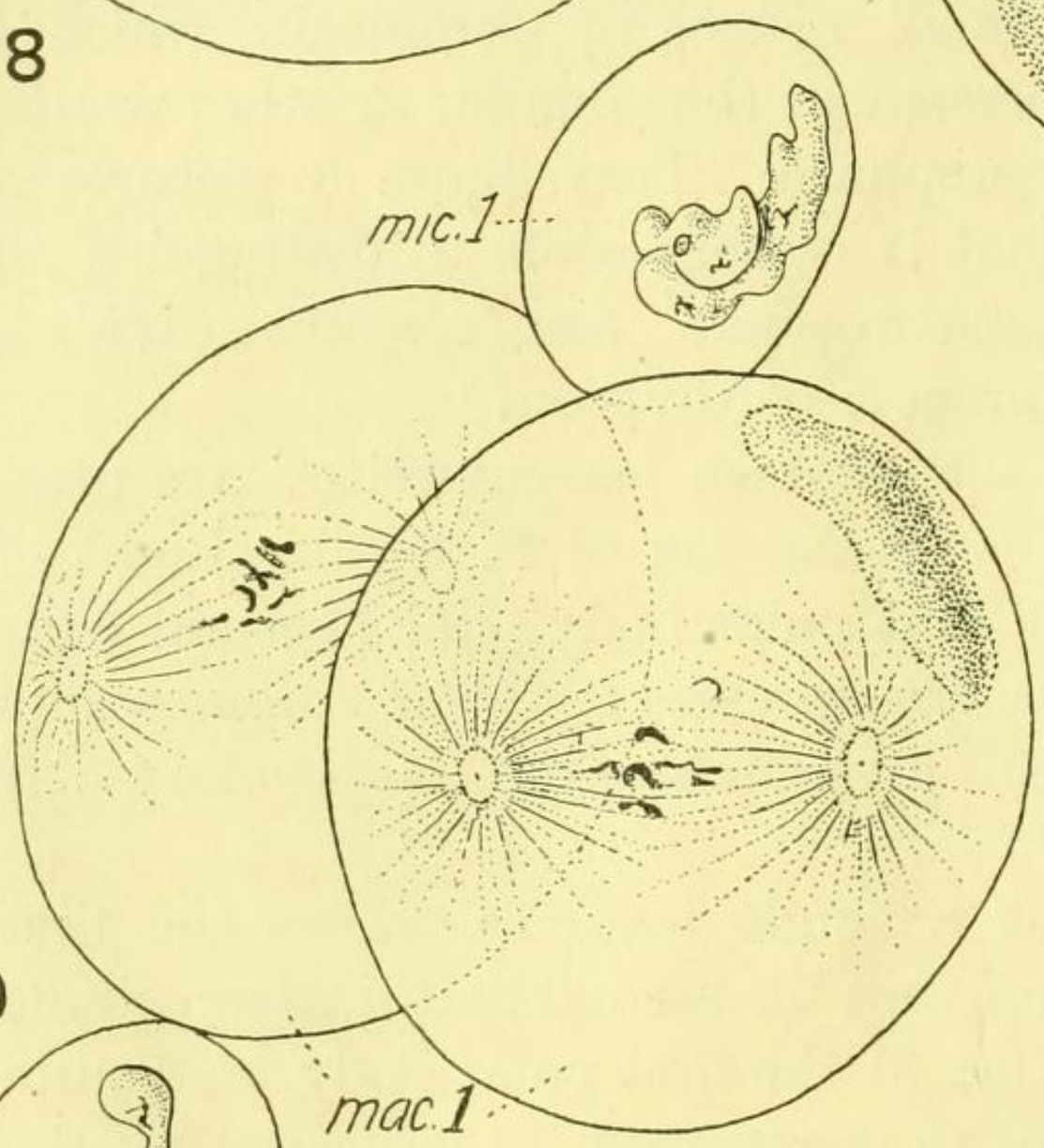
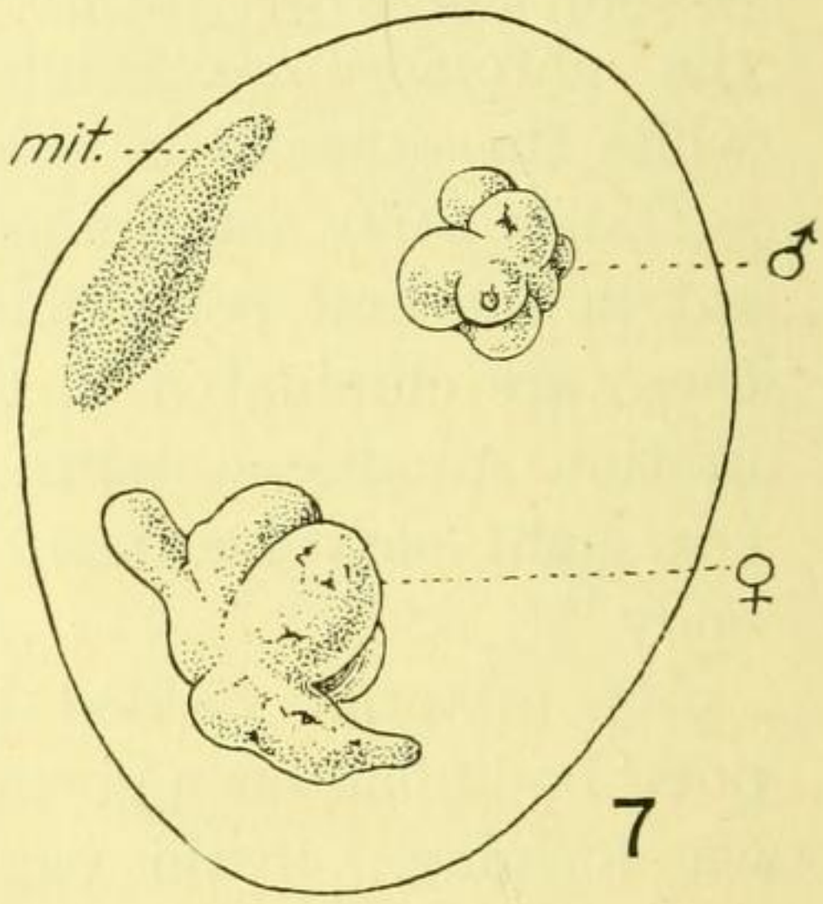

p.b.1
mit.
sp.

9


mic.1
mac.1

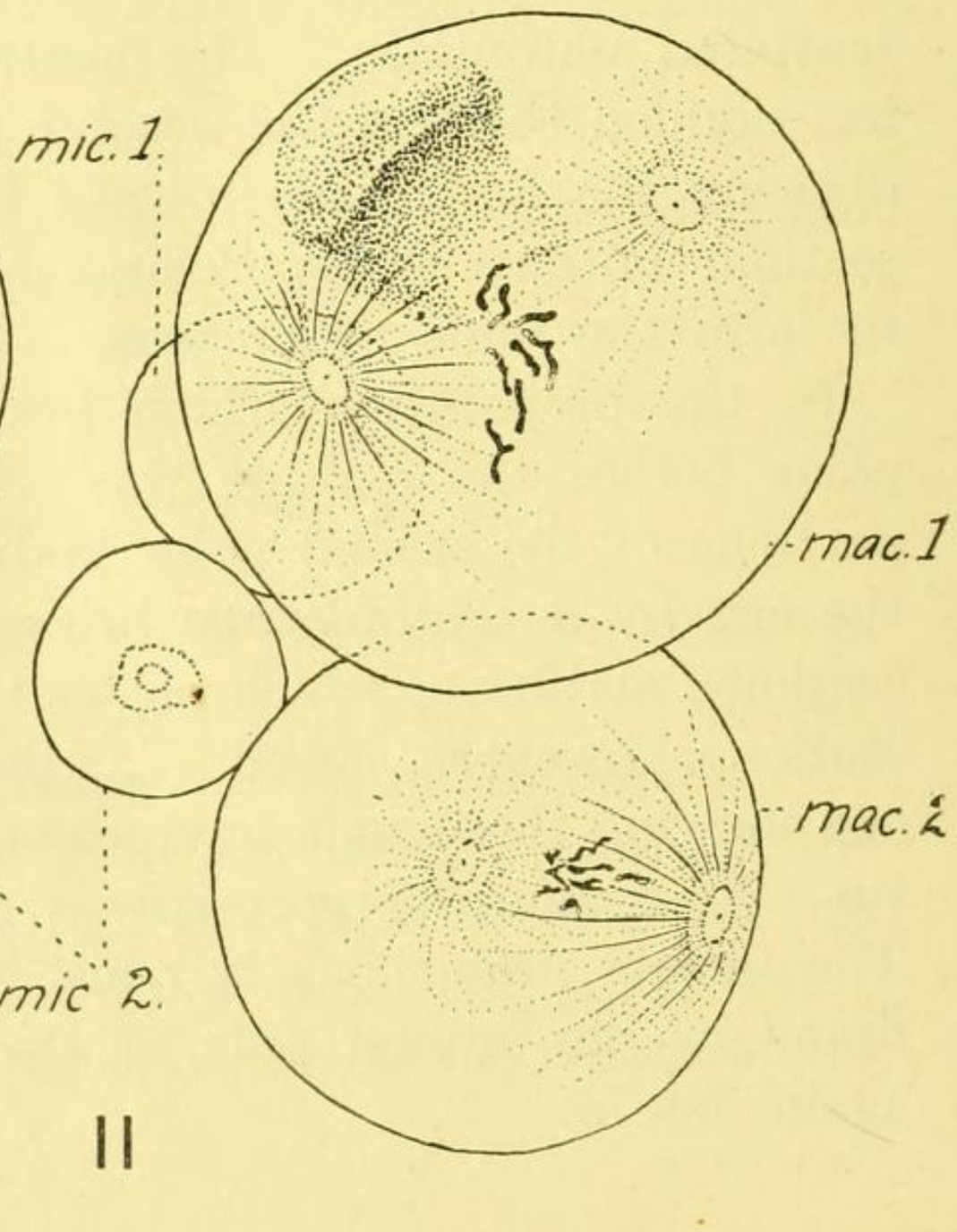
10


mit.
♂
♀

7


mic.1

mac.2
mac.1


mic.1
mac.1
mac.2
mic 2.

mit.

11

The first polar body at the left of the ovum has approximately twice the diameter of the second. That is it unmistakably smaller than the first two micromeres can be determined by comparing it with the first cleavage figures seen in text figures 10 and 11, while in text figure 12, drawn from life, the polar bodies and first micromere can be directly compared.

c. Fertilization. As stated above, insemination occurs before the onset of maturation. The point of entrance, illustrated by Patterson's figures and by text figures 6 and 9 of this paper, is somewhere in the hemisphere opposed to that from which the polar bodies are extruded. During maturation the sperm nucleus, after losing its flagellum, moves toward the center of the egg, at the same time enlarging to form the irregular male pronucleus.

Before their union both pronuclei become curiously amoeboid or lobular in form (text fig. 7). Mattieson ('04) noted this phenomenon in Planaria torva and P. polychroa, and Bresslau ('04) observed it in several Rhabdocoeles. The same was observed by Hallez ('09) in P. cardii.

The male pronucleus in text figure 6, having entered at some point near the vegetal pole, has penetrated the egg for about one quarter of its diameter. A distinct darkly stained mass of chromatin occupies the center of the otherwise chromatinless vesicle. Since, unfortunately, this preparation was rather heavily stained it is impossible to discern with certainty any degenerating remains of the sperm flagellum among the count-

Text fig. 7 Egg showing the lobulated male and female pronuclei. × 730.

Text fig. 8 Thick section of an egg containing the first polar spindle. One centrosphere has divided and the other is in the process of division. ♂ male pronucleus. × 730.

Text fig. 9 Section of an egg from the same capsule as the preceding. The first polar body is just being given off. *sp.*, spermatozoon; *mit.*, mitochondrial mass; *p.b.1.* first polar body. × 730.

Text fig. 10 Reconstruction from sections of a pair of embryos in both of which the first micromere has been given off. *mac.1.* first macromere; *mic.1.* first micromere. × 730.

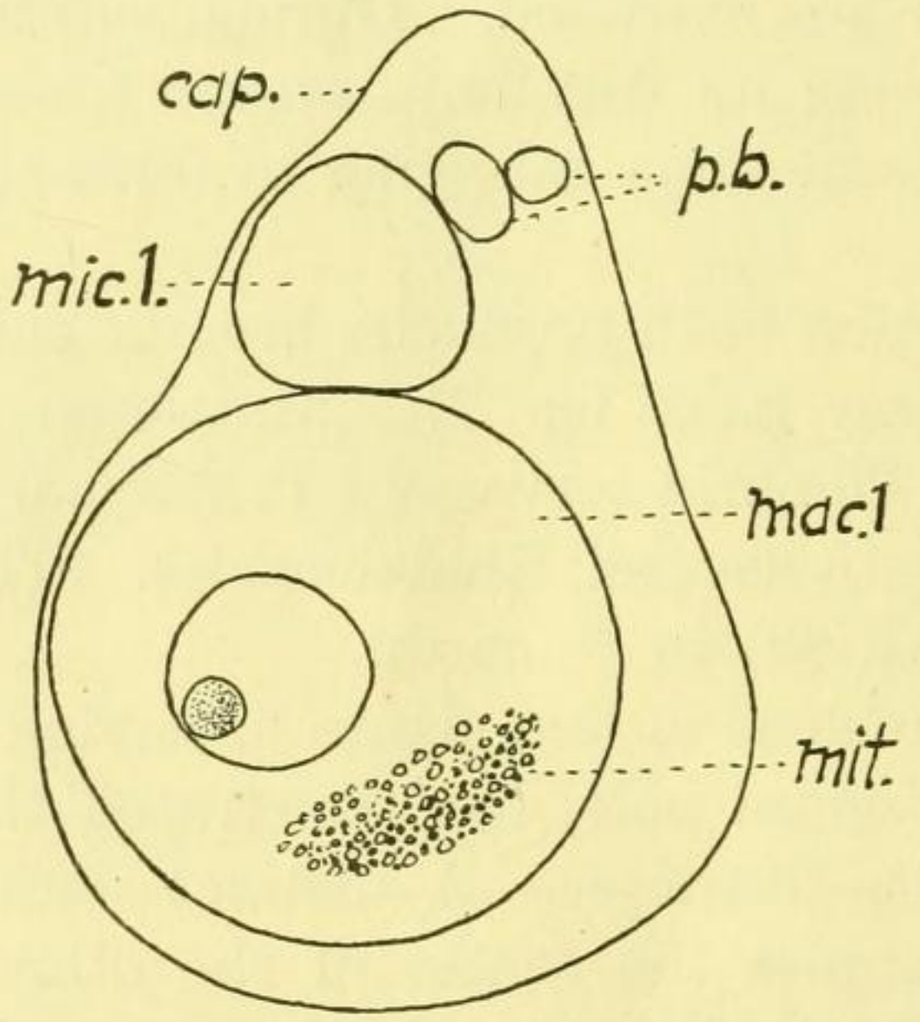
Text fig. 11 A pair of embryos from one capsule, each consisting of two macromeres and two micromeres. *mac.1*, *mac.2*, first and second macromeres; *mic.1*, *mic.2*, first and second micromeres. × 730.

less dark granules in the egg cytoplasm. It is plainly seen, however, in the earlier stages illustrated in text figures 4 and 9.

I can furnish no evidence concerning the union of the pronuclei; the most careful search failed to disclose any trace of centrosome and aster associated with the male pronucleus.

4. Cleavage

In the study of early cleavage in living capsules a serious difficulty has been encountered in the thick bed of yolk which



Text fig. 12 Outline from life of a young embryo observed in the antrum of the mother. *cap.*, capsular membrane; *mac.1*, first macromere; *mic.1*, first micromere; *mit.*, mitochondrial mass; *p.b.*, polar bodies. × 730.

surrounds the embryos. Text figure 12 was drawn from an egg, which, contrary to custom, had entered the antrum, become surrounded by the capsular membrane, and, since no yolk cells were enclosed, was conveniently easy to observe. If an attempt be made to remove the embryos at this stage they fragment. Late blastulae may be thus removed with some success (fig. 15).

The only alternative was a trial of various stains. As yet I have not had much success in staining the embryonic cells and at the same time leaving the yolk sufficiently transparent. Neutral red and methyl green were used to stain *in vivo*. Even

a dilute solution of the former acts very quickly on the yolk granules, so that no clear idea of early cleavage was gained by its use. Methyl green, a stain for living nuclei, left the yolk too opaque to allow any satisfactory determination of its effect on the embryo beneath. Acetic carmine and methyl blue, besides killing the embryo, again stain the surrounding yolk too strongly.

In later stages of development, after the embryos have migrated to the surface of the yolk or have taken it into their bodies, all the stains mentioned above can be used to advantage.

It is evident then that we must depend upon sectioned material for observations of the progress of cleavage and gastrulation. More or less uncertainty inevitably follows from the various degrees of contraction which the capsules undergo during fixation. (Hallez was partially successful in avoiding contraction by first placing the worms in sea water containing a little chloral-hydrate or cocaine.) The effect of displacement upon the embryonic cells is, of course, greatest in early cleavage. Earlier investigators have noted that particularly in Turbellarian development there is a very weak affinity between the neighboring blastomeres. Bresslau ('04) shows clearly in Plagiostomum girardi and that the blastomeres normally separate for distances often equal to their diameters; this phenomenon is evident as late as the twenty-four celled stage. Hallez's figures show in Paravortex cardii also a loose group of blastomeres. Later the cells draw together to form at first a rather loose, then a compact mass.

So in the case of P. gemellipara, although the blastomeres lie normally in fairly close apposition to one another, their mutual bonds are easily broken by outside pressure. As a result, sections often reveal isolated blastomeres crowded for some distance into the surrounding yolk.

For the reasons above noted it seems inadvisable to attempt any detailed investigation of cell lineage in this organism. Certain major characters, however, can be consistently observed.

a. Micromeres and macromeres. The first cleavage division of the egg of Paravortex gemellipara, as of P. cardii, gives rise

to a pair of cells between which there is a marked disparity in size. Text figure 10, a reconstruction of the two embryos of one capsule, shows very well the relative proportion in size of the first micromere to the macromere. It also indicates that gravity may not be sufficient to determine from which pole of the egg the micromere shall arise, for in this capsule the polarity of one egg is exactly opposed to that of the other. Not too much reliance, however, is to be placed upon the polarity as indicated in sectioned worms. When the animals are dropped into the killing fluid the tissues are as likely as not to become fixed while the specimens are inverted. Hence if the embryos rotate with respect to gravity, their positions with respect to the mother might easily be changed while the fixing fluid was penetrating her body-wall and parenchyma. But since both embryos are equally free to rotate, the evidence afforded by text figure 10 suggests that gravity does not wholly determine the embryo's polarity. Indeed, this figure, taken in conjunction with figures 14 and 18, indicates that the opposed polarity invariably exhibited by the two embryos of a capsule in later stages is already determined at the time of the first cleavage of the egg.

According to Patterson's account the first cleavage of the fertilized egg of P. gemellipara results in the splitting off of a micromere of such a size that it is necessary to count the chromosomes in order to distinguish it from the first polar body. We find, however, (text fig. 12), that this micromere is at least twice as large as the polar body, and furthermore (text fig. 10) that its nucleus immediately enters a resting stage during which it may be more or less amoeboid. No such nucleus has been found in the polar bodies. Owing to the rapidity with which the latter disintegrate, it is often impossible to find both the first polar body and micromere present together so as to compare them directly.

It is characteristic of the blastomeres of Paravortex gemellipara, as of P. cardii and other Turbellaria, that the nuclei are strikingly vesiculated.

Hallez asserts that in P. cardii the first micromere divides before the first cleavage of the macromere. Text figure 10

of this paper would indicate, on the other hand, that, since in both embryos the micromere contains an amoeboid nucleus while the macromere shows a spindle, the latter is about to divide before the first undergoes division. The second cleavage of the large macromere, judging from the conditions represented in text figure 11, results in the formation of two smaller macromeres somewhat unequal in size. The positions of the spindles which are inaugurating a further division of the two macromeres of both embryos indicate that the cleavage is to be of the spiral type described by Bresslau for Mesosotomum ehrenbergi. He found, however, that there were three micromeres present before the second macromere was cut off. Three of the macromere spindles in this pair of embryos are in the prophase, and clearly show eight chromosomes being drawn into the equatorial plate. On account of an artifact in one section of the fourth macromere the arrangement of the chromosomes could not be determined.

It is important to notice that during early cleavage the mitochondrial mass lies apparently inert at one side of the macromere and entirely outside the spindle. Not until later does the mass itself become divided.

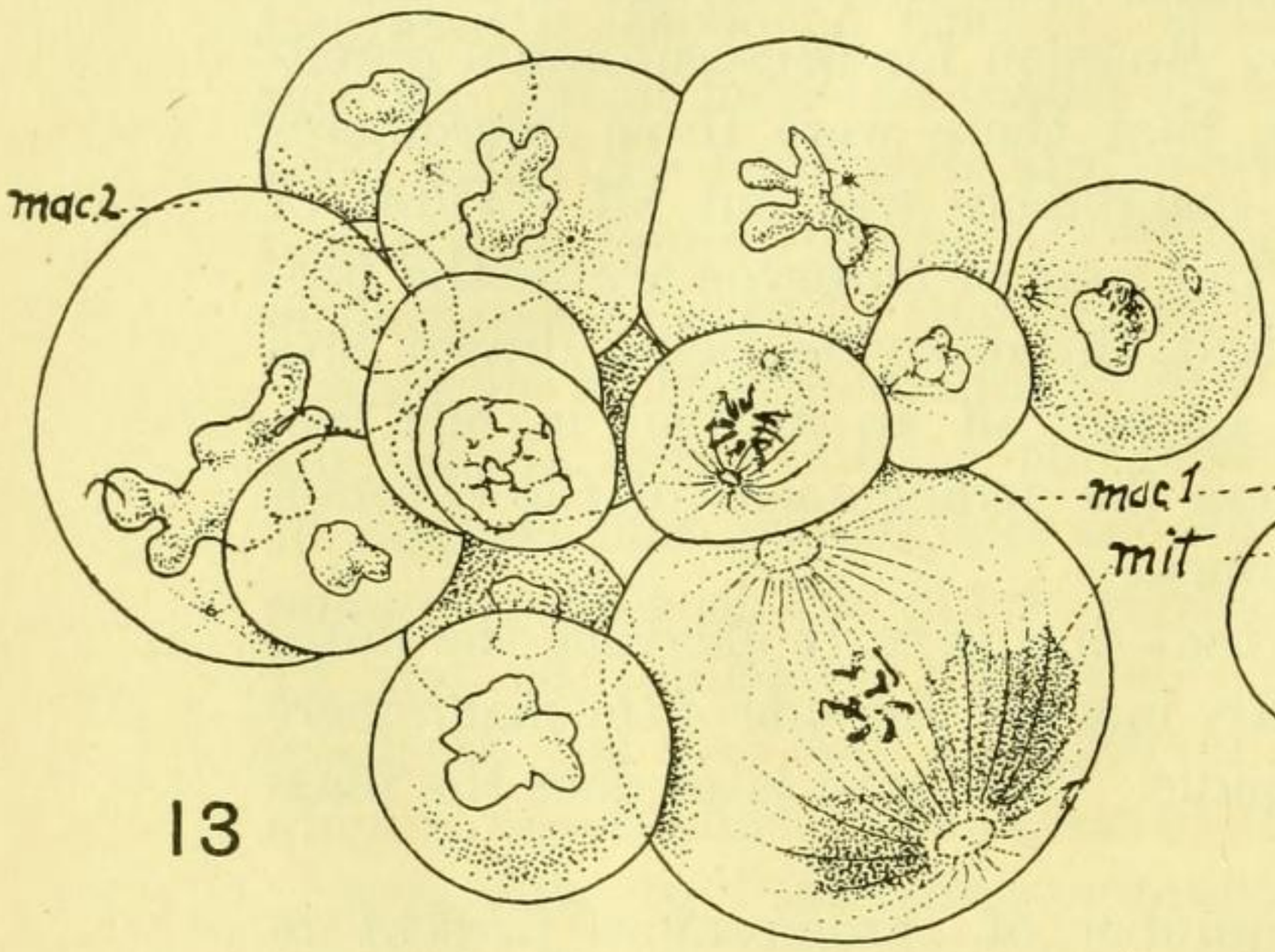
Hallez determined the number of chromosomes present in the equatorial plate of the cleavage spindle of P. cardii to be "four (= 4 groups binaires)." In P. gemellipara, however, the equatorial plate always contains eight U-shaped chromosomes; in the telophase each pole of the spindle likewise holds eight (text figs. 13, 14).

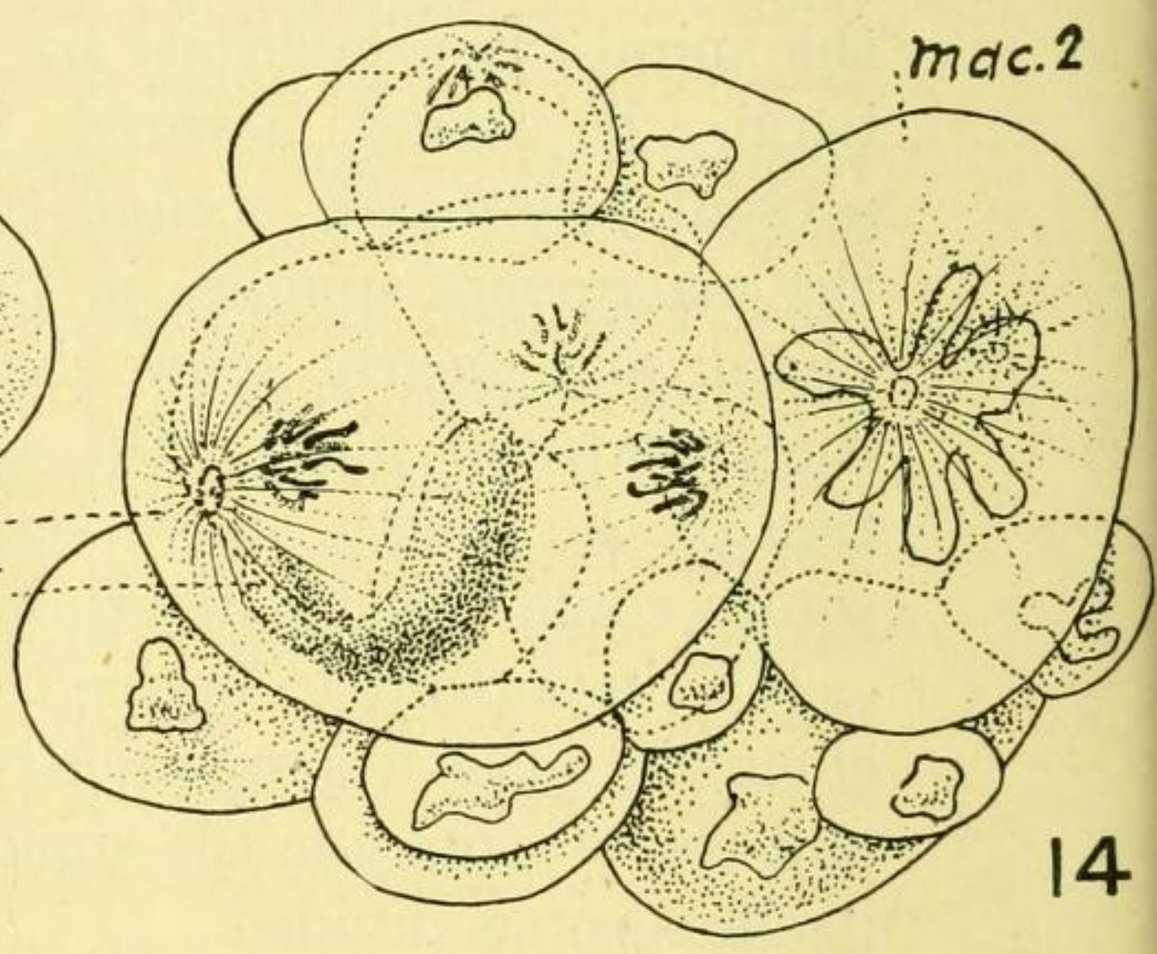
Beyond the four-celled stage cleavage proceeds irregularly as observed by Bresslau, Hallez and others. One can determine, however, that at one pole of the embryo the micromeres become more and more numerous; two macromeres occupy the other pole.

While the macromeres give rise by division to some of the larger micromeres, the latter redivide again and again.

The embryos represented in text figures 13 and 14 contained respectively fifteen and seventeen blastomeres. Text figure 14 is the more normal in that the group of cells is compact with-

out intercellular spaces. The embryo illustrated by text figure 13 had probably been subjected to a considerable degree of contortion. It is noticeable that the blastomeres in well preserved material are closely in contact with yolk, while in shrunken preparations wide spaces intervene; consequently the blastomeres are apt to become displaced. In both figures the pair of larger cells is prominent. The mitochondrial mass still remains in one of the macromeres. Text figure 13 shows it





Text fig. 13 Reconstruction from sections of an embryo of fifteen blastomeres. *mac.1*, *mac.2*, first and second macromeres; *mit.*, mitochondrial mass. × 730.

Text fig. 14 Reconstruction from sections of an embryo of seventeen blastomeres. × 730.

in the form of a hemisphere held at one pole of the spindle, while in text figure 14 it lies, cresent-shaped, outside the astral fibers.

b. Behavior of the mitochondrial mass. Although a careful search was undertaken, no cleavage figure was found in which the mitochondrial mass was first being divided. Between the stages shown in text figure 13 and in figure 20 no intervening step was discovered. However, the condition at a later stage, figure 19, suggests that, as the membrane is drawn inward during cleavage of the macromere, the mass is too large to remain wholly in one cell and is so constricted that part is handed

on to each of the daughter cells. It is possible, on the other hand, that the division is effected directly by the traction of the spindle fibers upon the mass. Very likely both factors act simultaneously.

Certain it is that beyond the stage where the mitochondrial-mass appears in two or more blastomeres the centrosome lies in its center. Previously the centrosome was difficult to detect among the numerous granules in the cytoplasm, but now that it has come to lie in a more finely homogeneous substance it is conspicuous. Figures 14 and 16 plainly show it in a clear region at the center of the mitochondrial mass. Had its history not been carefully traced the picture here presented would suggest that the whole structure answered to the centrosphere containing a centriole. That the black dot represents more than a mere centriole is indicated by its further behavior. Figure 19 shows that the solid dot becomes enlarged as the new spindle forms, and contains a light center. Ultimately the body assumes the form of a sphere whose shell consists of a multitude of tiny granules, and at whose center lies the true centriole. Furthermore, it lies outside the mitochondrial mass in one cell of figure 20.

c. Blastula. It has been noted that no distinct segmentation cavity is formed in the blastula. The conditions at this stage are well shown in figures 14 and 16 which represent sections of different embryos, and in figure 15, a drawing of a living embryo which, with a small mass of yolk granules, was freed from its capsule. The blastomeres lie in rather close contact with each other, with the exception that a cap of cells which have received the portions of the original yolk-nucleus tends to become separated from the mass of embryonic cells which is to give rise to the living tissues of the embryo. A solid morula is, then, typical of P. gemellipara as of Turbellaria in general and many other lower invertebrates. But for the cells containing the mitochondrial mass this morula closely resembles that of Hydra and other Coelenterates. Bresslau figures for Mesosotomum ehrenbergi a morula somewhat less compact and more irregular in outline.

His figures of the morulae of Bothromesosotomum personatum and Mesostomum lingua are similar, with slight characteristic variations. Although the blastomeres in the segmentation of the egg of Plagiostomum girardi separate remarkably from one another, Bresslau finds them later drawing together to form a compact mass.

The morula of P. gemellipara lacks the marked bilateral symmetry due to a sagittal cleft which, according to Bresslau, characterizes a corresponding stage in the development of Mesostomum ehrenbergi. In P. gemellipara it has been found impossible to distinguish the dorsal from the ventral surface of the morula. The point of view in figure 15 is from almost directly anterior; any attempt to distinguish here between dorsal and ventral is clearly of no avail; the cells are all alike. On the lower left, however, the posterior primary entoderm cells are differentiated by their larger size.

d. Homologues of the germ layers. While it is true that, as Bresslau says of Mesostomum and other Turbellaria, no definite germ layers are formed in these worms, at least rather distinct germ regions are discernible. As the development of the morula progresses there are produced as derivatives of the one macromere about sixteen blastomeres which receive a portion of the mitochondrial mass. They form a cup-like layer, one cell thick, over the surface of the morula which lies toward the center of the yolk-mass, and serve, as will soon become clear, to indicate the polarity of the embryo; this cap of cells is posterior to the rest of the morula, and is to be looked upon as constituting the primary entoderm. Two other regions can be distinguished in the morula at this stage, (fig. 16), first, the mes-ectoderm consisting of smaller cells which are conceived to be direct descendants of the micromeres, and second, a layer of larger cells with less dense cytoplasm lying between the primary entoderm and the mes-ectoderm. These are derivatives of the second macromere, seen in text figures 11 and 13, which lacked the mitochondrial mass. Since they ultimately give rise to the intestine it is appropriate to call this layer of cells the secondary entoderm.

In the slightly older embryos represented in figures 17 and 18 the secondary entoderm cells are still actively dividing, but most of them are larger than the anterior mes-ectoderm cells.

5. Absorption of yolk by the primary entoderm

Toward the end of the morula stage a remarkable process of nutrition is inaugurated. As far as has been determined from the literature no closely similar process has been described as occurring in the nourishing of any embryo. The primary entoderm cells, which were seen in figures 14 and 16 already to have but a loose connection with the main embryonic mass, now apply themselves like amoebae to the surface of the yolk which surrounds the posterior half of the embryo. The firmness of their attachment is evident in figure 17; while the embryos have shrunken away, most of the yolk entoderm cells remain closely in contact with the yolk.

It is the function of these cells to absorb a large part of the yolk mass brought into the capsule inside the vitelline cells. The membranes of the latter have all disintegrated, leaving the yolk flakes and granules free in the capsule. Inspection of the living capsule shows the particles in vigorous Brownian movement. There is, therefore, no obstacle to the action of the entoderm cells. They are shown in figure 17 plainly acquiring the yolk particles. Their nuclei are large, distinct, and according to the rule in active cells throughout this animal, markedly lobulated.

The nature of the yolk granules suffers little change during the process, for those inside the entoderm cells have the same form and color as those lying just outside the cell membrane. It is to be noted that all exist as spherules; no flakes such as constitute a considerable portion of the free yolk are at first seen in the entoderm.

The gradual increase in size of the entoderm cells, while ingesting the yolk on the median side of the embryo, at the same time forces the latter outward until that portion of it which from now on is to be recognized as the anterior end comes to

lie in contact with the wall of the capsule (fig. 17). This migration of the embryo from an interior to a peripheral position is a step characteristic of all the species considered in this paper. According to Bresslau the embryonic cells migrate to the ventral surface of the yolk in the species of Mesostomum and Plagiostomum which he studied. Hallez ('09) finds it assuming an antero-ventral position in Paravortex cardii. The same migration was observed in the Dendrocoeles by Mattieson ('03) and in the Triclads by Jijima ('84). The embryonic mass of Paravortex gemellipara, like that of P. cardii, is forced to the anterior side of the yolk.

There is considerable variation in the amount of yolk absorbed by the primary entoderm (compare figs. 18 and 20). Hence there remains a larger or smaller quantity disposed, as seen in a median section (fig. 17), in the form of a bi-concave lens between the two embryos. The fate of this mass will be discussed in the following section.

Some of the entoderm cells seen in figure 21 have already reached the limit of their absorptive capacity, while others are still comparatively small. It should be pointed out, however, that there is a great variation in size at the time they are taken into the embryo. That the quantity of yolk absorbed depends rather upon the activity of the cell than upon the amount of yolk in its neighborhood is suggested by the fact that small primary entoderm cells are enfolded by the gastrulating embryo even when there was plenty of material present, as indicated by the quantity later found in the ectoderm.

At the completion of the yolk absorption, and before gastrulation, degeneration of the nuclei of the primary entoderm cells sets in. As represented in figure 21, their chromatin becomes less distinct, while the nucleoli disappear. Finally, with the dissolution of the nuclear membranes, there remain for a time only indistinct vestiges of nucleoplasm with a little chromatin. Rarely does any trace of the nucleus appear in the cell after it has been taken into the body of the embryo. The activity of the cell having ceased, the presence of the nucleus is no longer necessary.

6. Ingestion of the yolk-gorged primary entoderm cells by those of the secondary entoderm

A remarkable event now happens. Some of the derivatives of the secondary entoderm cells pointed out in figures 14 and 16 become amoeboid and make their way around and between those of the primary entoderm until they come to lie, in some cases, next to the free yolk. Three of these are visible in each embryo of figure 18. Though the nuclei are always prominent, it is only in well-preserved and favorably stained material that the cytoplasm can easily be distinguished. In such cases there can be no question of a syncytium here. Each cell is a unit.

Another amoeba-like character is now manifested by these cells; each primary entoderm cell is ingested by a secondary entoderm cell. Referring again to figure 21 we find that the section passed through the nuclei of several secondary entoderm cells, while the cytoplasm of each stretches in a thin sheet over the surface of the primary entoderm cell which is being engulfed. There is in this embryo no certainty that the cytoplasm has yet completely surrounded the yolk-filled cells. That this occurs later will become clear. It is evident that the nuclei of the primary entoderm cells do not entirely disappear until after ingestion.

7. Gastrulation and absorption of yolk by the ectoderm cells

If by the process of gastrulation is meant the infolding or overgrowth of the entoderm by the ectoderm, then a true gastrulation occurs in Paravortex gemellipara. Bresslau ('04) in describing the development of Mesostomum thought the term inapplicable. It is used in this paper as expressing the process at least homologous with gastrulation.

The first step consists in the differentiation of the ectoderm. The outermost cells of the mes-ectodermic mass at two points (or perhaps in a ring) on the anterior surface of the embryo now become flattened. Between these two points lies a pair of cells which are to give rise to the lining epithelium of the pharynx

(figs. 22 and 23). Just as Bresslau finds in Mesostomum so in P. gemellipara the ectoderm cells stretch backward in a sheet over the underlying mass (fig. 21). Figure 22, drawn from a section so thick that only the upper third of the embryo was removed, represents the condition just before the close of gastrulation. The five yolk cells, three of which show the large nuclei and thin cytoplasmic sheets of the secondary entoderm cells whose food they have just become, are being folded into the embryo by the overgrowth of the ectoderm cells. At the right three of the latter are of such a character that they resemble the yolk cells just described.

It is apparent from the above account that gastrulation in P. gemellipara is brought about by a process of epiboly or overgrowth of the entoderm by the ectoderm cells. The latter are formed by differentiation of the external portion of the mesectodermic mass, whereby the cells, first at the anterior end, then gradually those lying more and more laterally, become flattened and push backward over the entoderm.

At this point another phase in the nourishing of the embryo is observed. It was noted above that, after the gorging of the primary entoderm cells, more or less yolk remained in the capsule (figs. 18 and 21). The ectoderm cells, now undertaking the role of nutrition, absorb this free yolk. In figure 22 the cells on the right still show the yolk granules distinctly, indicating that the process here is similar to that carried on by the entoderm. Very soon, however, the yolk undergoes a change. The flakes and granules in each cell flow together into a single large globule. It is evident from this figure that the posterior cells take in most of the yolk, those at the anterior end coming in contact with only a very little. The nuclei of the ectoderm are characterised by the presence of large and distinct nucleoli.

It is strikingly evident that in embryos of corresponding stages of development, particularly that shown in figure 24, the ectoderm yolk-content of some is decidedly less than of others. The conclusion is that the primary entoderm absorbed the greater part. According to this hypothesis but little yolk would have

entered the ectoderm cells of the embryo pictured in figure 18. Such individuals as those in figures 22 and 23 must previously have had left over after the absorptive action of the entoderm an amount of free yolk comparable with that shown between the embryos of figure 21.

a. The embryo at the close of gastrulation. The picture presented by the embryo at the close of gastrulation is shown in median frontal section by figure 23 which was drawn from an Ehrlich's haematoxylin-eosin preparation. Sections of seven primary entoderm cells occupy the greater part of the young worm, each lying inside an amoeboid secondary entoderm cell. No clear regions have yet appeared in the interior of the former; the yolk globules are evenly distributed.

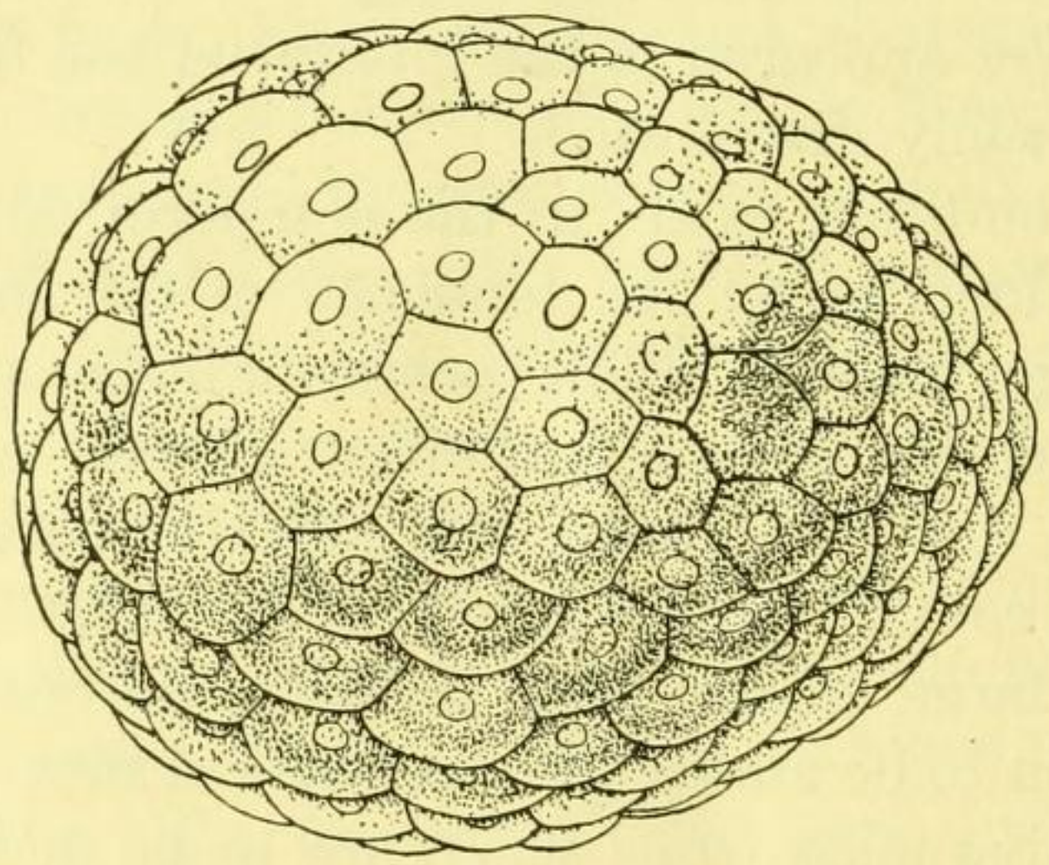
A similar condition exists in the ectoderm, although a slight advance is evidenced by the tendency of the small globules to fuse into larger masses. The posterior cells are so engorged that their membranes are stretched almost to the point of rupture. On the other hand the anterior ectoderm cells are flattened and contain only a few yolk granules. It is probable that certain large nuclei just beneath the ectoderm in this region are soon to be added to it as parts of new cells.

The formative mass from which are to be derived all the definitive organs lies toward the anterior end of the embryo. Two lines of cells, however, are pushing posteriorly beneath the ectoderm and the primary entoderm. Certain of these are undergoing mitosis, while a few differ from the majority in possessing lighter cytoplasm and definite boundaries. These are regarded as secondary entoderm cells which did not migrate outward to ingest the yolk-laden cells, but were reserved to take a part in the formation of the intestine.

It is impossible in the section from which figure 23 was drawn to detect cell membranes between the smaller nuclei which characterize the greater portion of the undifferentiated mass. One might well regard it as a syncytium were it not that the membranes are discernible in figures 18 and 21 which represent slightly younger embryos, and in figure 24 of one somewhat

farther advanced. These three figures are from preparations stained with iron-haematoxylin and eosin after fixation in Flemming's solution.

To conclude the description of the stage seen in figure 23, the pair of cells mentioned above (fig. 22) as being reserved during the early period of ectoderm differentiation for the development of the internal pharyngeal epithelium, are conspicuous at the anterior end of the embryo. Their cytoplasm remains consistently lighter and clearer than that of the adjacent interior cells. Their resemblance is rather on the side of the ectoderm.



Text fig. 15 Surface view of an embryo forcibly freed from its capsule shortly after gastrulation. Anterior end at the right. × 530.

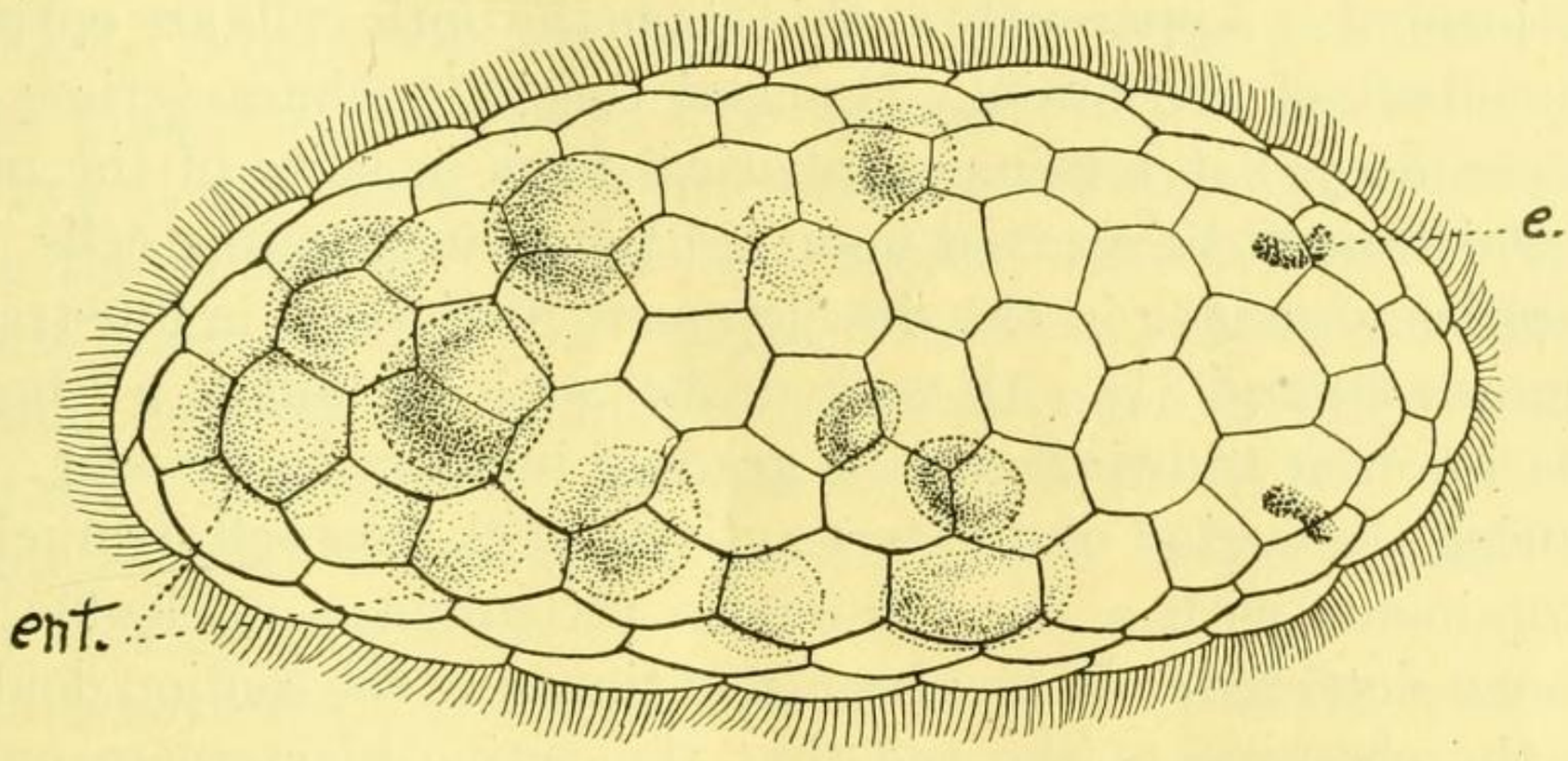
Figure 25, drawn from a fresh capsule, shows the appearance of a living embryo at a stage slightly later than that in section (fig. 23). Through the transparent ectoderm can be seen the prominent yolk-filled cells of the primary entoderm. The highly refractive bodies so conspicuous in the ectoderm cells are regarded as globules of the same nature as those constituting the free yolk still remaining in the capsule. The larger opaque masses in the posterior ectoderm cells are the homologues of those noted above in figure 22, i.e., masses of yolk undergoing digestion.

Between the capsular shell and the enclosed embryos and yolk granules is a clear zone filled with a colorless fluid. Prob-

ably a large part of this clear region is occupied by a fluid of a watery nature which has been absorbed from the parenchyma. It was pointed out above that capsules which are freed in sea water immediately swell on account of pressure due to water absorption.

8. Organogenesis to the time of birth

a. Origin and development of the eyes. The first organs to be differentiated from the mes-ectodermic mass in Paravortex gemellipara are the eyes. Although at first this seems rather



Text fig. 16 Surface view of an embryo which of its own account had made its way into the mother's parenchyma. *ent.1.*, digested remains of yolk in primary entoderm cells; *e*, eye. × 400.

remarkable, still it is reasonable to expect that, since the major portion of each definitive eye consists of a single cell, this cell must at an early stage take on a different appearance from those about it. Already in figure 24 the eye cell shown has become distinct by its larger size and clearer cytoplasm.

These eye cells appear, one on either side toward the dorsal side of the undifferentiated mass. That seen in figure 24, an oblique frontal section, is an individual cell with a distinct membrane (Hallez was unable to distinguish the membrane in P. cardii) and nucleus. The latter at first lies in the center of the cell, but soon assumes an eccentric position toward the posterior median side. The presence of only a single melanin granule is certain on account of the fact that the whole cell is included in

this section. Moreover, the second eye of this embryo was in the same stage.

The fate of these undifferentiated cells will be treated later. The eyes are at first separated from the ectoderm by three or four rows of cells. Partly as a result of increase in size which the eyes undergo, and partly because the cells among which they lie are gradually withdrawn to form other tissues, the eye cells shortly assume a position so close to the ectoderm that only one layer of nuclei intervenes. The left eye in figure 28 (from a frontal section through the eyes and brain) has become finally located. Apparently at this stage the optic cells are entirely independent of the brain. Unusual turgidity characterises the embryonic eye; it retains a spherical form in spite of the pressure which must be exerted upon it by the surrounding cells.

A series of stages in the development of the eye is illustrated by figures 30 and 31. All were drawn with the aid of a camera lucida in order to bring out the gradual increase in the size.

During the period of greatest activity of the eye cell its nucleus is prominent, with a fine chromatin reticulum; a nucleolus has not been detected. It has been mentioned in the section dealing with the cleavage of the egg that the active blastomere nuclei tend to assume an amoeboid form. So in the eye cell the nucleus becomes to a lesser extent irregular during the deposition of melanin granules.

Two types of melanin spherule deposition have been observed. The more usual is that wherein the granules arise one at a time. While those first formed are increasing in size new ones appear in the cytoplasm (fig. 30). The earlier melanin bodies are formed close to the lateral surface of the nucleus. Hence it might be inferred that, being laid down under the influence of the latter, these would soon be pushed outward by new ones to be formed in the same proximity to the nucleus. Such a sequence, however, has not been observed; the new spherules arise as often on the outside of the group as on the inner nuclear border. Indeed, after the process has gone on for some time practically all the smaller granules are seen to lie on the side opposite the nucleus.

As to the general arrangement exhibited by this group of melanin spherules, the early irregularity soon gives way to that of a cup whose concavity faces outward (fig. 30 *d*, *e*). Consequently a meridianal section is crescentic. The floor of the cup, lying against the nucleus, consists of from two to four rows of larger granules, while as the rim is approached the thickness is gradually reduced to one small sphere.

According to the second type of melanin deposition many minute particles arise simultaneously in the cytoplasm between the eccentrically placed nucleus and the outer membrane of the eye cell. Figure 31 represents the appearance and history of these granules. Since the final arrangement is the same as in the eye at the end of the series described above, it appears either that the spherules nearest the nucleus increase more rapidly in size while those at the margin of the cup remain consistently smaller, or that new granules have subsequently been added to this rim. The latter hypothesis is supported by the fact that a larger number of spheres is present in the definitive eye than was first seen to arise.

It seems clear that the pigment granules arise in the cytoplasm, probably under the influence of nuclear energy. Their arrangement and appearance are not visibly affected by the method of preparation; hence they can be favorably studied in preserved material. When first detected each is a tiny homogeneous refractive body whose form is that usually assumed by a drop of fluid whose surface tension properties are different from those of the fluid in which it is suspended—in this case cytoplasm. There is no evidence that these spherules enlarge by the accretion of successive layers of a more solid material. The conditions rather favor the idea that they grow in the manner observed in the case of fat drops, i.e., by an accumulation of more and more fluid.

With the increase in size the yellowish color deepens to a reddish brown, and a darker central dot appears in each granule (fig. 30 *c*). From this point on there is little to be noted beyond the increasing opacity due to the greater amount of material. Finally, when the pigment spheres have reached their full size

their color is an intensely dark brown (fig. 30 *e*). At low magnifications the pigment cup is to all appearance black.

The nucleus of the eye pigment cell is gradually forced by the melanin granules against the membrane at the postero-median side. Figure 32 shows it thus flattened. In proportion to the size of the cell the nucleus is now smaller than during the period of its greatest activity.

b. Development of the brain and larger nerve trunks. Bresslau ('04) determined that the formative mass in the embryo of Mesostomum ehrenbergi soon separated into three anlagen, a large antero-ventral mass, consisting at first of two ganglia which later formed the brain; a spherical portion just posteriorly from which the pharynx musculature arose; and third a smaller cresentic gonad anlage.

Hallez ('09) noted the similarity exhibited to this stage of Mesostomum by the corresponding stage of Paravortex cardii. In the development of P. gemellipara the differentiation of the brain, pharynx musculature, and gonads is essentially the same as in the species studied by Hallez. In two laterally symmetrical regions toward the dorsal side of the anterior embryonic mass a proliferation and grouping of nuclei occurs soon after gastrulation. Thus the two ganglia of the brain arise. While the nuclei become more and more numerous, it has been impossible to discover any mitoses in this region, a fact which led Hallez to suggest that division here is amitotic.

The second feature noted in the development of the brain is the appearance simultaneously of two clearer protoplasmic areas in the center of each lateral ganglion. The substance occupying these two regions has been called 'Punktsubstance' by Leydig. As pointed out by Bresslau and Hallez, these two centers soon fuse to form a transverse dumb-bell shaped core on the outside of which the ganglion cell nuclei are arranged. The latter are at first indistinguishable from those of the surrounding portions of the undifferentiated cell mass, but in slightly older stages are visibly smaller (figs. 29 and 53).

Concerning the 'Punktsubstance' mentioned above one characteristic is to be noted that neither Bresslau or Hallez described

in either text or figures, namely, the striated or fibrillar structure of the lighter brain core when viewed in sagittal sections. That these striations are to be regarded as the fibers or prolongations of the ganglion cells whose nuclei are distributed over the surface of the brain, is clear from the works of many investigators. These structures are well represented in Bronn's Klassen und Ordnungen des Thier-Reichs. Obviously it is these fibers which, in a cross-section of the brain, i.e., a sagittal section of the worm, are responsible for the dotted appearance. Figures 29 and 37 are of sections so cut that some nerve fibers are seen in cross and others in longitudinal section.

Little remains to be added in regard to the nervous system. Since the material was not treated with differential nerve stains, it is extremely difficult to follow the nerve trunks through the mesenchyme, even in older specimens.

Concerning the origin of the two posterior nerves of Paravortex cardii Hallez says:

"Quant aux deux troncs nerveux postérieurs, tout ce que j'ai pu voir, c'est qu'ils se différencient *in loco*. . . ."

It is believed that in P. gemellipara these posterior nerves can hardly be said to arise '*in loco*.' They evidently push backward through the mesenchyme from the posterior angles of the brain. In figure 28 the nerve fibers can be traced thus for a very short distance, but in a later stage (fig. 29) the ends have reached farther toward the posterior end.

It is important to notice that many ganglion cell nuclei are carried backward along the nerve trunks. Consequently the cephalic mass suffers a reduction. From the conditions observed it is concluded that in the Turbellaria, as in the vertebrates, the nerve fibers, with groups of ganglion cells, originate in the region of the brain and migrate toward the periphery.

That the optic nerve trunks have a similar origin may be argued from the appearance presented by figures 28 and 37. However, these nerves may have another origin. The fibrous brain core in figure 28 has begun to stretch forward at two antero-lateral points; a decided advance has taken place at the stage pictured in figure 32. Here the left lobe of the brain and the

left eye are viewed from the dorsal surface. The nerve fibers show very plainly passing from the 'Punktsubstance' region of the brain outward and between the ganglion cells. The course of the nerve is then directed laterally around the posterior side of the pigment cell to its external surface.

At this point the eye cell, whose origin and growth have already been described, undergoes an invagination. This is apparently effected by the pressure of a nerve cell which is seen lying in the concavity. This undoubtedly will form the 'sight-cell' or end organ which is stimulated by light rays. A second nerve cell nucleus is observed just posteriorly.

It can now be suggested that the two cells lying close outside the pigment cell in figure 32 arose in this position and have sent fibers backward to meet the anterior extension of the brain. Two facts support this view. First, undifferentiated cells during development lie in this region between the ectoderm and the pigment cell. Secondly, it is well known that in higher forms the cell body of the receptor is located in the retina, while the axone extends into the brain. It is obvious, however, that one must not attempt to homologise too closely the course of development of a turbellarian with that of a vertebrate. In the latter the retinal cells are first carried out by the optic cup from the central nervous system, while in the Turbellaria the boundaries of cell groups in the cephalic region are notably indistinct.

It has proved impossible to discern in my material the structure of the cell or cells which occupy the external invagination of the pigment cell in the adult worm. Since the histology of the eye in related Turbellaria has been determined, it is to be expected that the receptor of P. gemellipara is similar to that observed, for instance, in Phaenocora and Mesostomum as shown in Bronn's Klassen und Ordnungen des Thier-Reichs.

Figure 33 of this paper was drawn from an adult eye of P. gemellipara, and shows that the optic nerve, as in the embryo and in the species to which we have just referred, passes to the lateral invaginated surface of the pigment eye-cell. A nucleus rests close outside, apparently in the nerve.

c. Pharynx. Simultaneously with the differentiation of the brain the anlage of the pharynx musculature becomes marked off from the former and from the remaining tissue by a closer grouping of larger nuclei. This mass lies ventral and very slightly posterior to the brain, but its boundaries are at first so indefinite that, were it not for the simultaneous ingrowth of the cells which form the internal pharyngeal epithelium, the anlage would with difficulty be distinguished.

Considerable attention has been paid by European observers to the development of the pharynx of the Turbellaria. Mattieson in 1904 described the process in the Dendrocoele, Planaria torva, in which the pharynx arises as a spherical mass of cells on the ventral surface just beneath the primary ectoderm. While the outer cells form the musculature of the organ, the internal epithelium arises from a few large cells situated on the interior. Against the inner end of the pharyngeal mass lie four or five cells which in Mattieson's opinion are entodermic in nature. No vestibule intervenes between the pharynx and mouth.

Bresslau ('04) found that in the Rhabdocoele Bothromesostomum personatum the internal epithelium likewise arises *in loco.* In Mesostomum ehrenbergi, a different condition exists; the lining epithelium is derived from a rod of cells which grows inward from the ectoderm and pierces longitudinally the mass which eventually becomes the pharyngeal musculature with its glands. After traversing the pharynx the ectodermic rod acquires a lumen and gives rise at the inner end to what Bresslau terms the inner pharngeal pouch lying between pharynx and yolk, while between the pharynx and mouth an outer pharyngeal pouch has a similar origin.

The main features in the development of the pharynx in Paravortex gemellipara agree with those described by Bresslau for Mesostomum ehrenbergi. Until after the ingrowth of the epithelial rod no definite grouping of nuclei occurs to mark the origin of the pharyngeal bulb. The cells which are to take a part in its formation lie loosely arranged in the mid-ventral portion of the undifferentiated cell mass just beneath and posterior to the brain.

In the description of the ectoderm formation there was pointed out a pair of cells which, although at that time lying at the anterior surface of the embryonic mass, did not enter into the development of the body covering. These two cells, well shown in figure 23, together with two or three others not seen in this section proliferate the rod of cells which, as in Mesostomum, grows inward to penetrate the mass which lies beneath the brain.

An intimate connection between these cells and those of the ectoderm is evident in figures 34 and 26. The former represents a section which is obliquely sagittal. It is evident that before the cells of the pharyngeal bulb have become closely grouped, the posterior cells of the epithelial rod have penetrated the mass of dark nuclei and lie against the entoderm. A distinctly clearer cytoplasm and paler nuclei serve to distinguish them from the neighboring cells. Each of their nuclei, like those of the ectoderm, with whose early history these cells are closely associated, contains a nucleolus. Externally a slight invagination of the ectoderm indicates the future position of the mouth.

A slightly later stage is shown in figure 27, a transverse section through the posterior lobes of the brain and the center of the pharynx. Portions of seven clear cells belonging to the epithelial rod are conspicuous in the center of the mass of nuclei which have now become closely grouped with their cytoplasm in what appears to be a syncytium. For a longitudinal view of the organ at this stage the obliquely frontal section in figure 26 is of interest. The nuclei of the bulb are disposed roughly in two rows, somewhat toward the periphery of the mass. As yet no cell membranes appear between them. The epithelial cells are constricted within the bulb so that they become elongated, parallel with the longitudinal axis of the organ; one cell appears to traverse its entire length. Those between the pharynx and epidermis are irregularly arranged, while at the inner end of the pharynx is a group of five similar cells. Three of these appear to be intimately connected with the epithelial rod; the posterior pair, however, are not thus closely associated, but have, it is believed, an entodermic rather than an ectodermic origin. Further

notice of these cells will be taken when the development of the intestine is considered.

In an older embryo (fig. 37) the muscular portion of the pharynx has become sharply defined, although no external epithelium has yet developed. The nuclei have not migrated farther toward the periphery than the point at which they were observed in the preceding stage. A considerable enlargement of the anterior and posterior cells of the epithelial rod has occured. No blind sac, however, is formed, as in Mesostomum, at either end of the pharynx. Indeed, no lumen appears in the pharyngeal apparatus until shortly before the embryo leaves the mother's body.

If at this stage the pharynx and associated tissues of Paravortex gemellipara be compared with the corresponding stage in Mesostomum ehrenbergi it is evident that the development of the pharynx in the former lags behind that in the latter. Mesostomum shows a well defined epithelium bounding a large cavity, the latter consisting of an internal and external pocket communicating by a tube through the pharynx. Bresslau considered the interior pocket as the esophagus, whose cells differed in point of origin from those later forming the intestine. The homologue of this pocket in P. gemellipara is the group of large cells just posterior to the pharynx.

In the cross section of this organ represented in figure 38 an advance is shown in the presence of an external epithelium. A thin layer of plasma containing a few small flattened nuclei covers the pharyngeal bulb. Bresslau derived this tissue from the outermost portion of the pharyngeal musculature anlage in Mesostomum; but in Paravortex the indications point to a mesenchymatous origin, such as von Graff ascribed to it in other Turbellaria. When one remembers that in either case the cells constituting this membrane arose in the undifferentiated cell mass the point resolves itself into one of time.

Concerning the development of the radial, circular and longitudinal fibers which arise in the pharyngeal musculature nothing has been observed in the young of Paravortex gemellipara while still in the mother's body.

d. Musculature. Like the nervous system and the pharynx the mesenchyme and dorsoventral muscle cells arise from the undifferentiated mass which occupies the anterior portion of the embryo at the close of gastrulation. If one compares figure 23 and figure 24 it is noticed that in the latter indifferent cells have migrated posteriorly so that a continuous sheet of them lies between the ectoderm and entoderm. Their amoeboid nature is further evidenced by the extreme delicacy of their membranes. Indeed, it is impossible to distinguish a boundary where two cells come in contact with each other. The tissue at this stage may be called a syncytium. Occasional mitoses are observable in this region. The plane of the section of the embryo at the right in figure 24 is sagittal, and somewhat toward the left side of the body, and the dorsal margin lies toward the top of the page. Thus it appears that in P. gemellipara the migration of cells occurs as freely in the dorsal as in the lateral and ventral portions of the embryo. In the later stages, however, as Hallez pointed out, there are comparatively few nuclei on the dorsal side of the entoderm (fig. 37).

There are two types of nuclei in this sub-epithelial layer, an external row of small rather deeply stained nuclei which usually lie against the inner surface of the ectoderm cells, and an internal series of larger and paler nuclei, some of which are slightly irregular. The second type is more numerous on the lateral and ventral surfaces. The embryo at the left was tangentially sectioned, thus showing the more lateral nuclei.

Passing to the more advanced stage shown in the sagittal section in figure 37, where the brain and pharynx have become differentiated, it is found that certain of the cells containing the smaller nuclei have become spindle-shaped. The cytoplasm has formed a process at each end. These spindle cells are still more conspicuous in figures 27 and 36.

Bresslau ('04) describes similar cells at a corresponding period in the development of Mesostomum as representing the early stages in the differentiation of the dorso-ventral muscle fibers. The number observed just beneath the ectoderm of Paravortex rather suggests that from these cells arise the circular and longi-

tudinal muscle fibers which soon become so conspicuous in this position (fig. 40). These sub-epithelial muscle cells of the adult Rhabdocoeles have been observed by many investigators. The figures in Bronn's "Klassen und Ordnungen" show the remarkable extent to which they become fibrillated.

In Paravortex as in most of the Rhabdocoeles, a muscle net is developed just beneath the epidermis. Figures 40 and 41 show the outer layer of circular and inner layer of longitudinal fibers in the adult condition. Since the figures in the various works which have been accessible never show nuclei in connection with the fibers of this muscle net, a vigorous attempt was made to find the cells from which these fibers arise in the embryo of Paravortex gemellipara. Of interest in this regard is the cell shown in figure 39, for it is possible to trace several long delicate fibers outward from the cytoplasm surrounding its nucleus. The latter lie close beneath the external epithelial cells in the position later occupied by the muscle net. Coe ('99) traced such a development of the muscle fibers in the Nemerteans Micrura caeca and Cerebratulus marginatus.

If this interpretation of the origin of the muscle fibers be correct, then a further question presents itself. Do these fibers later become independent or do they maintain a connection with such a cell body as is seen in figure 39? It is difficult to conceive, judging from the parallel arrangement of muscle strands in the adult, that the fibers are still in connection with these cell bodies. The latter, moreover, cannot be detected in the adult tissue. The conclusion is, therefore, that either the fibers become separated from the nuclei, or that the latter by repeated division are so reduced in size that they escape observation.

e. Sex organs and vitellaria. Not until after the young leave the mother's body does the true differentiation of the sex organs commence. At the time the young worm breaks out of the capsule and enters the surrounding mesenchyme the anlagen of the two hermaphroditic glands are only loose masses of cells, one on either side just lateral and posterior to the pharynx. In one animal which had forced its way into the mother's intestine a few cells of these paired anlagen were undergoing mitosis,

while those of the neighboring tissues were entirely without mitotic figures (fig. 49).

Just previous to this stage of development occasional large cells make their appearance in the ventral region of the body between the entoderm and the layer of muscle fibers. These cells are represented in figures 37 and 43 as having larger, clear nuclei and a considerable amount of darkly stained cytoplasm. The color of the latter in iron-haematoxylin preparations is a bluish gray.

It is believed that these large cells later develop into the yolk cells of the vitellaria. The grounds for this conclusion will be brought forward in the section on post-embryonic development. But their behavior while the young worm is still enclosed in the capsule may be traced somewhat farther. Since they appear first in the region close behind the sex organ anlagen it is probable that they arise, either directly from them or from cells which have had a similar ancestry. It is known of other Turbellaria that the vitellaria become differentiated from the same mass with the ovaries and the testes (Hallez '09). So in Paravortex gemellipara, which at the height of its reproductive activity is characterized by the presence of remarkably extensive vitelline glands, the general rule holds concerning their origin; but in point of time their branching off from the common sex organ mass seems unusual.

f. Cilia. The epithelial cells become furnished with cilia shortly after the stage seen in figure 34. They are already present in figure 35, but of their development nothing has been observed beyond the fact that, although they are as long when first noted as in older worms, they are not nearly so numerous as on the adult epithelium. The embryos at this stage are able to move about in the capsules. The ciliary action can easily be observed through the maternal tissues when the living worm is studied.

g. Embryonic digestion and early development of the intestine. In an earlier section of the paper it was explained that the gastrulating embryo, like those of all Rhabdocoeles, enclosed within itself the free yolk material which had previously entered the capsule as a constituent of the vitelline cells. The greater

portion was absorbed by primary entoderm cells which were in turn ingested by amoeboid secondary entoderm elements. The ectoderm grew backward over the entoderm and at the same time absorbed the remainder of the vitellarial yolk. Figure 23 was pointed to as illustrating the appearance of the embryo at the close of gastrulation. By far the greater portion of the embryo is occupied by the yolk-laden cells.

The fate of this yolk now remains to be described. In the stage represented by figure 23 little change has occured in the nutritive material. It is of the same nature as the free yolk seen in figure 21, with the exception that the flakes and globules are less closely packed in the cytoplasm of their containing cells. There is observable, however, a tendency of these particles to flow together into larger globules, a process which is more rapid in the ectoderm cells. Thus in the two epithelium cells at the top of figure 23 several such spheres are conspicuous.

In an older embryo (fig. 34) this sphere formation has advanced considerably. The spherules of nutritive substance combine again and again until the major portion is included in one or two immense globules. That the yolk is undergoing a transformation is indicated by the appearance of darker spherules suspended in the large masses. These smaller elements stain dark brown or black in iron-haematoxylin preparations such as figure 34 represents.

In figure 25 is illustrated the appearance of the ectodermic nutritive material in the living embryo after treatment with the neutral red stain. The latter has a more rapid effect upon the larger masses than upon the smaller. After immersion for a very few minutes in a dilute neutral red solution it is impossible to see through the posterior ectoderm; all is a mass of deeply stained red spheres.

Returning to figure 34 it is seen that clear regions have appeared in the interior of each entodermic yolk cell. These regions in the iron-haematoxylin-eosin preparations are perfectly colorless, but in those treated with Ehrlich's haematoxylin and eosin after fixation with corrosive acetic, they take the eosin color (figs. 27, 36). Thus it is evident that these lighter portions are

filled with a plasma. To how great an extent the separation of the yolk-filled contents into two zones is due to the action of reagents has not been determined. It is interesting to note in this connection that most of the spherical yolk-mass enclosed in the embryo in figure 42, an Ehrlich-stained specimen, is homogeneously filled with yolk-granules such as originally entered the cells. Still this embryo, as indicated by the presence of the cilia, is more advanced in development than the one illustrated in figure 34.

One entoderm cell, that at the upper left in figure 42 demands attention. The yolk material which it contains has entirely assumed the fluid nature seen in central regions of these cells (fig. 36). Now it has been explained that the Flemming-iron-haematoxylin-eosin method leaves these regions entirely colorless. Hence the cell now under consideration, if stained by this latter method, would have apparently contained a vacuole in the position here occupied by the yolk mass. Exactly such a condition is exhibited in figure 37. In this embryo there appear what at first were regarded as vacuoles within a mesenchymatous network; they are now rather to be interpreted as the transformed yolk material accumulated within the entoderm cells as immense globules which, following the corrosive-acetic Ehrlich's haematoxylin-eosin method, stain a rich yellow. It has been pointed out that by this method also the nuclei, cytoplasm and membranes of the entoderm cells containing these nutritive masses are much more clearly stained than by the iron-haematoxylin.

Another feature to be noted in the entodermic yolk masses in figure 42 is that the mitochondrial mass is again discernible. This body was traced from its origin within the oocyte through cleavage to its division and enclosure in the primary entoderm cells. During the early history of the mass the Ehrlich stain was unfavorable to its demonstration, since the cytoplasm of the cell retained so deep a stain that the mitochondrial mass was obscured. But in the late stage shown in figure 42 the cytoplasm of the original cell has broken down and the granular mass of mitochondria again comes into prominence. The centrosome

with which it was earlier associated may still be detected, although less easily than during the period of its activity.

A certain fact favors the view that a process of disintegration has attacked the mitochondrial substance. For whereas, up to the time when the entoderm cells had absorbed a considerable amount of yolk, the body appeared conspicuously in iron-haematoxylin material, it has been found impossible to detect it in such preparations at later stages of development. This peculiarity, taken in connection with the ability of the Ehrlich stain to bring it out subsequently, points to the conclusion that the mitochondrial substance has become chemically changed. Furthermore, no trace of it is to be observed after the yolk globules have been transformed into such a fluid mass as noted in the upper entoderm cell of figure 42. It would be too extreme to surmise that the mitochondrial mass represents a substance laid down in the oocyte for the purpose of acting as a digestive agent after the yolk has been taken inside the embryo. Such an interpretation would have to regard the substance as a potential ferment, which, before it could act upon the yolk, must undergo a transformation which renders it invisible.

Against the view that this mitochondrial mass is functional at this stage is the evidence furnished by the ectoderm. Here a simultaneous yolk-digestion has been in progress where none of the mitochondrial substance entered. In figures 35 and 37 each posterior epithelial cell contains one or more of the large fluid spheres. In appearance and reaction to the Ehrlich's haematoxylin-eosin stain, these masses are closely similar to those in the entoderm. As we have seen, their origin also is the same. In the iron haematoxylin preparations, however, the ectodermic material stains brownish while the entodermic masses are colorless. It is probable that both substances are lipoids of some sort.

After the young worm has left the capsule and is prepared to take food through the mouth the reserve material present up to this time in the ectoderm rapidly disappears. The process is apparently one of gradual absorption and assimilation. In one preparation a cell just beneath and closely applied to the

ectoderm contained two small globules, of the same consistency and color as the food material in the entoderm. It is possible that through the agency of mesenchymatous cells the ectodermic nutriment is passed on to the tissues of the embryo. On the other hand this cell may have belonged to the entoderm, its contents representing the last stage in the digestion of the entodermic yolk.

During the absorption of the reserve food, the ectoderm, or external epithelium of the young worm, has undergone vacuolization; the cytoplasm of each cell now forms trabeculae between irregular clear regions (fig. 37). This condition is at first more noticeable at the anterior end of the body where less yolk is deposited, but with the absorption of the latter posteriorly the whole ectoderm takes on this appearance. This vacuolization causes a marked increase in the depth of the cells.

h. Formation of the intestine. According to Bresslau ('04) the intestinal epithelium is formed in Mesostomum by previously undifferentiated embryonic cells which unite first in the region just posterior to the esophagus. Gradually this sheet of cells, by the addition of others, extends posteriorly about a cavity which arises as a splitting of the tissues in the region of the fast shrinking yolk cells. At the same time the intestinal cells become vacuolated.

Hallez ('09) asserted that the cells which enter into the formation of the intestine of Paravortex cardii are of the same nature as those which give rise to the mesenchyme. He considers their immediate predecessors to be large cells lying at the posterior end of the body. These proliferate at once cells which are added to the intestinal 'syncytium,' and others which constitute the mesenchyme between the intestine and body wall. He observed that a lumen appears first in the region just behind the oesophagus, and gradually, with the absorption of the vitelline spheres, extends posteriorly.

From certain observations of the conditions under which the intestine arises in Paravortex gemellipara there is some doubt that it originates in the manner described by Hallez for P. cardii. It seems rather that the large posterior cells, as noted in the

preceding section on the sex organ and vitellarium anlagen, ultimately differentiate to form the vitelline cells. Their development will be considered later.

In P. gemellipara the intestine is organised from cells which, at the stage illustrated by figures 35 and 36, are proliferated in the region just posterior to the oesophagus. Two of these in figure 26 were pointed out above while the pharynx was being considered. It is believed that they are derivatives of the large clear cells designated as *ent. 2* in figure 23.

With the rapid absorption of the yolk inside the secondary entoderm cells, the latter become enclosed with the epithelial cells which, arising in the manner above described, migrate backward about and between them. In this way a loose rod of cells comes to occupy the whole region behind the pharynx. As Hallez observed, the intestinal lumen first appears at the anterior end of the rod, and gradually extends posteriorly. As long as the young worm remains in the capsule there is no communication with the outside through the pharynx and mouth. It is probable, however, that it is potentially present, requiring only the muscular activity of the pharynx to open the lumen. The young animals, when liberated from the mother by pressure upon the cover-slip, exhibit contraction and expansion of the pharyngeal apparatus, a feature which indicates that, as soon as they are in a position to obtain food from without, they are provided with a lumen from mouth to intestine.

i. Glands. Shortly before the young worm leaves the capsule from two to four elongated oval or spindle-shaped bodies appear between the brain and the vertex of the anterior end of the body (fig. 45). In iron-haematoxylin preparations these masses are grayish in color, while Ehrlich's stain imparts to them a deep red. In animals which have entered the tissues of the mother these gray bodies communicate with the exterior (fig. 46) by slender processes; they are now recognizable as the cephalic glands. To these has been attributed the function of secreting a slime which lubricates the animal's path.

Numerous other spindle-shaped cells distributed through the sub-epithelial tissue are evidently single-celled glands which have to do with the secretion of mucous.

9. *Post-embryonic development*

In order that the further development of the sex organs, intestine, mesenchyme and glands could be studied, an attempt was made to keep alive in small aquaria young worms which had been removed from adults, into whose parenchyma they had already become freed. No success was met with; all died inside of seventy-two hours.

Therefore several young of the smallest size obtainable from the mussels by ordinary methods were sectioned in the hope that some of the organs would be found in an undeveloped state. While this trial gave some interesting results, it is desirable that a study be made of specimens younger than these and at the same time older than those secured at the time of birth.

The following observations are submitted as being of interest in this connection.

a. The definitive intestine. Hallez describes and figures the adult intestine of Paravortex cardii as consisting of a veritable syncytium, a mass of cytoplasm containing large vacuoles and scattered nuclei. The intestinal wall of P. gemellipara, however, consists of elements which are distinct except at their extreme bases. Figure 47 was drawn from a worm which measured 0.30 mm. in length. The intestine, having already assumed the adult condition, is seen to consist of large cells which extend from the outer surface of the organ inward to its lumen. Their bases are constricted and narrowed, while the distal ends assume a rounded form where they extend into the intestinal cavity. Numerous vacuoles, some of them containing food materials, are distributed throughout the cytoplasm. Most of the nuclei are located in the narrowed ends of the cells. Figures 8 and 9 exhibit a similar appearance of the intestine of old worms. A like structure of the turbellarian digestive tube is figured in such works as von Graff's Monograph and Bronn's "Klassen und Ordnungen,"

b. Mesenchyme. According to Hallez's ('09) account of P. cardii, the mesenchyme receives elements, both from the undifferentiated cell mass at the anterior end of the body and from the large cells which become grouped at the posterior extremity.

Bresslau ('04) also derived the mesenchyme from the undifferentiated cells.

The origin of this tissue in P. gemellipara agrees with that described by these investigators except that there is no evidence of a contributory action on the part of the large posterior cells; it is all derived from the undifferentiated cell mass, from which cells migrate posteriorly between the intestine and body wall. The mesenchyme of the free-swimming young worm from which figure 47 was drawn already existed in the condition which is characteristic of the adult. The cells are large and loosely arranged with indistinct and irregular boundaries.

c. Ovaries and testes. The youngest stage in the development of the sex glands of P. gemellipara in which they can be recognized is shown in figure 47. Although the testis lies close to the anterior end of the ovary, the two glands are already separate. No epithelium, however, has been formed about them. Since this specimen had been stained with Ehrlich's haematoxylin, and since the section was so thick that the entire testis and nearly all of the ovary are included, the minute condition of the chromatin cannot be determined. The small dimensions of the nuclei in comparison with those observed in the adult ovary indicate that all the cells in the female gonad, except possibly the most posterior, are oogonia.

An important feature in this specimen is that the vitellaria have become conspicuous, particularly in the posterior half of the body. No connection between these glands and the ovaries can be traced in this series of sections.

The next instructive stage which my material presented is shown in figure 50, a sagittal section through one testis, the seminal vesicle, atrium, shell-gland and the tissue which is to form the antrum. In the testis the spermatogonia and spermatocytes can easily be distinguished. Hallez described and figured a similar appearance for the young testis of P. cardii. One feature of which, however, he failed to give an account is that the gland becomes provided with a flat epithelium. The latter is derived from the mass of cells lying below the mid-ventral side of the intestine. From this same mass the accessory reproductive

organs are also being differentiated. It is evident that the seminal vesicle arises from the anterior portion of the mass; only a small number of cells at the extreme anterior end go to form the vasa deferentia and the external epithelium of the testis.

In the region ventral and posterior to the seminal vesicle the atrium has differentiated simultaneously. Like those of the former, its walls are composed of two layers of cells, an inner cubical epithelium and an outer sheet of flattened cells. As yet no connection of its lumen with the outside of the body has been effected. Indeed, the cells are not even in close contact with the body wall.

From the posterior surface of the atrium a dense mass of cells leads a short distance dorsally. The antrum femininum will arise later in this position. On the ventral surface of the mass is a group of cells whose rounded posterior ends have begun to reach backward behind the atrium. These cells enlarge to form the single-celled shell-glands.

Of the oviducts nothing is as yet present but a loose string of undifferentiated cells which lead from the antrum anlage posteriorly and dorsally to the ventral surface of the ovary. It was impossible to distinguish about the latter such an epithelium as was described for the testis, nor is it evident in later stages.

The ovaries in the worm from which figure 50 was drawn are well advanced. The older oocytes have undergone a considerable growth.

Figure 51, from a somewhat larger worm, is presented to show clearly the strand-like structure of the testis and the point at which the vas deferens enters the seminal vesicle. The oogonia occupy the anterior region of the testis. These, by growth, give rise to the large primary spermatocytes lying on the ventral and dorsal sides; while in the center of the posterior half a row of smaller, densely stained cells are regarded as secondary spermatocytes. No spermatids were distinguished with certainty, but mature spermatozoa are conspicuous in the anterior central region whence they can be traced dorsally between the cell strands to the upper surface of the testis. Others lie just be-

neath the external epithelium and in the vas deferens, while many have already entered the seminal vesicle. Thus it is evident that Paravortex is protandrous to the extent that spermatozoa are produced before the female reproductive tract has become differentiated, for no lumen has appeared farther backward than the atrium.

d. Vitellaria. In the section wherein the earliest anlagen of the sex organs were described it was noted that several large cells with an unusual quantity of darkly staining cytoplasm and conspicuous nuclei were observed to arise in the region of the gonads and to migrate posteriorly between entoderm and ectoderm (fig. 43). While these cells became distributed on all sides of the latter, a marked accumulation occurs at the extreme posterior end (fig. 44). Hallez was of the opinion that these cells in P. cardii contributed largely to the formation of the intestine, but also to the mesenchyme and vitellaria. There is no evidence that they give rise in P. gemellipara to any other organ than the vitellaria. These cells are identical in appearance with the young cells which lie at the extreme tips of the vitelline strands in the adult. Figure 44 shows at once the embryonic cells and, just outside the capsule, several of the young vitelline cells in the mother's tissue.

By the time the young worm has attained the stage represented in figure 47 strands of cells similar to those of the adult have grown forward from the posterior mass; at the same time the scattered cells of the same type have divided to form groups here and there through the mesenchyme of the posterior region. At *x* two of them are undergoing mitotic division; similar figures have been observed at the tips of the adult vitellarial strands. As yet very few have appeared in proximity with the ovaries. Hence there seems to be no evidence that in P. gemellipara the vitelline glands develop, even in part, from the hermaphroditic gland as it appears at this stage; these organs lack all connection with any other densely stained tissue. In the reconstruction (fig. 52) from an older worm, however, the vitellaria have formed a contact with the posterior ends of the ovaries. The various groups of yolk cells have anastomosed to form an extensive gland.

V. DISCUSSION

In the preceding account of the development of Paravortex gemellipara little attention has been paid to the interpretation given by Hallez ('09) of the processes of yolk absorption and the origin of the ectoderm in P. cardii.

He maintains that, while the morula is being formed by the cleavage of the egg, the membranes of the yolk-cells included in the capsule disintegrate, but that a part of their nuclei remain prominent and active. Hallez finds these nuclei shortly afterward lying in a clear plasma-like portion of the yolk which has become separated from an eosin-staining constituent and has assumed a peripheral position outside of and between the embryos. On the other hand the 'eosinophile' portion of the yolk separates, he believes, because its chemical and physical properties are different from those of the cytoplasmic remainder. As these properties become more pronounced, the central yolk material splits off in the form of great drops in whose interior he finds a variable number of vacuoles. He has figured several nuclei, which are assumed to have been set free by the disintegrating yolk cells, lying in the outer clear zone; others have migrated inward between the embryos. As pointed out earlier in this paper, he found that one of these nuclei became associated with each of the 'balles vitelline.'

Even more remarkable is Hallez's account of the developmental phenomena which immediately follow. Into each vitelline sphere one of these migratory nuclei penetrates, together with a small quantity of the cytoplasm which also was previously a constituent of the vitellarium cells. At the same time the material of these yolk spheres assumes a granular appearance.

A little later these nuclei, he states, emigrate with their associated cytoplasm from the 'balles vitellines.' Part of these amoeboid cells are asserted then to mingle with the envelope of plasma outside the embryo so as to form a 'syncytium' from which a primary ectoderm soon differentiates. Other such cells delaminate from the yolk spheres as additions to the ectoderm. Into the formation of the latter now enter, as Hallez believes,

the remaining vitellarial nuclei which did not previously penetrate the yolk spheres. Hallez observed also that the true embryonic cells contribute to the development of the ectoderm. This ectoderm is retained permanently. The remaining 'migratory cells' form another 'syncytium' about the vitelline spheres which they have just left, this constituting, in Hallez's opinion, the primary intestine.

The ectoderm on the apposed surfaces of the two embryos is formed by a delamination through the center of the yolk-cell derived plasma with its migratory nuclei. Hallez discerned but few cell membranes.

From a study of his figures and descriptions it seems possible that owing to a failure to distinguish the extremely delicate outlines in the embryonic structures he has been led to the above interpretation. I believe that further study may show that the processes of early development in Paravortex cardii are very nearly the same as those which I have found in Paravortex gemellipara.

The derivation of the ectoderm and entoderm as observed in the development of P. gemellipara agrees essentially with that determined by Bresslau for the several species of Rhabdocoeles which he studied. A variation in the manner of yolk absorption, however, occurs in Paravortex. Whereas he found the yolk cells either to retain their envelopes, as in Mesostomum ehrenbergi, or to be enclosed by the ectoderm in the form of numerous yolk spheres resulting from the breaking down of the vitelline cells, in P. gemellipara the yolk granules are first absorbed by the primary entoderm cells, and these are in turn ingested by the secondary entoderm. The first step of the process is similar to that known to occur in the chick, where the intestinal entoderm absorbs the yolk particles.

That the yolk-absorptive function of these entoderm elements is their chief capacity is further indicated in a freak embryo shown in figure 53. This specimen, beside lacking the yolk-filled masses so characteristic of normal embryos, exhibits a remarkable distribution of the undifferentiated tissue. The latter has proliferated at a normal rate, so that following its

posterior migration it has become arranged many rows thick between the ectoderm and a few large clear cells in the center. The latter are believed to belong to the primary entoderm which ordinarily absorbs the yolk; in this case it is probable that no food was enclosed with the egg in the capsule.

The behavior of the entoderm cells in P. gemellipara is comparable to that observed in 1884 by Lang in the Polyclad Discocoelis. Since the egg of this worm contains a rich supply of yolk material, none is added in the form of vitellarial cells. During segmentation an upper and a lower quartet of entoderm cells arise whose derivatives later form the wall of the alimentary canal. These entoderm cells have a more or less amoeboid character, sending out protoplasmic processes over the yolk spheres. The latter were previously split off from the yolk-filled middle entoderm which takes no part in the formation of any organ.

In 1907 Surface described the development of the alimentary canal in Planocera inquilina. He found that practically all of its tissue arises from two large entoblast cells. By division they form two sorts. One group containing the yolk is pushed into the interior of the embryo where the cells break down and the yolk granules flow together into a fluid mass of large spheres. Meanwhile the lower entoblast cells divide rapidly, thus giving rise to a large group of cells. A cleft, the first indication of the intestinal lumen, now occurs in this entodermic mass, and cell outlines become distinct about the nuclei. The important feature to be noted in relation to the behavior of the entoderm of P. gemellipara is that Surface observed these cells to become amoeboid and to spread out on the surface of the large yolk spheres which are thus absorbed.

Now it is only a short step from the condition described by Surface in a Polyclad to that which I have observed in the Rhabdocoele, Paravortex. Here two entoderm elements—the large macromeres of text figure 14—give rise by division to the primary and secondary entoderm cells. The former, since they contain no yolk of their own, absorb it from the vitellarial substance. Like the upper yolk cells in Planocera, their nuclei degenerate and their membranes break down. But this latter

step occurs only after they have been ingested by the secondary entoderm cells, which thus resemble in behavior the cells of the alimentary canal in Planocera.

Hallez states that in P. cardii no membrane is formed over the surface of the yolk mass by a part of the vitellarial cells. P. gemellipara resembles P. cardii in this respect; no shell-membrane arises. But Hallez thought he found a similarity between the behavior of the yolk-cell nuclei of Paravortex and Mesostomum in that certain of these nuclei in the former played an important part in the differentiation of the definitive ectoderm and primary intestine; according to Bresslau, in Mesostomum ehrenbergi, the peripheral yolk cells form a shell membrane over the surface of the remaining yolk. In Paravortex gemellipara, however, every cell which enters into the structure of any organ is a derivative of the egg. The vitellarial yolk-cell nuclei and membranes disintegrate, leaving only the yolk as food; the nuclei never contribute to the formation of living tissue.

Of the mitochondrial mass little remains to be said. Its history from the first appearance in the young oocyte was traced until it finally disappeared in the entoderm of the young worm. The suggestion was tentatively made that the body might be or might produce an enzyme which has to do with the transformation of the yolk. But a similar transformation took place in the ectoderm, a tissue which received none of the mitochondrial mass. It therefore appears that the literature concerning the 'Dotterkern' has received another contribution, but without further explanation of its action. Truly suggestive, however, is the behavior of the body.

VI. SUMMARY

1. The origin of two or more embryos found in the capsules of Paravortex gemellipara is similar to their origin in other capsule-forming Rhabdocoeles; that is, by the enclosure of two or more eggs and about one hundred yolk cells in a shell secreted by the shell glands.

2. The mitochondrial mass (yolk-nucleus) was traced from its origin in the young oocyte through cleavage to its final resting

place in the several primary entoderm cells. Its function probably has to do with the absorption and digestion by these cells of the vitelline yolk. The behavior of the mitochondrial mass is different from that observed in other animals in that, whereas in most forms it either disappears in the egg stage or, if remaining through cleavage, is restricted to one cell, in P. gemellipara several cells ultimately receive a portion of the original substance.

3. Three germinal regions are recognizable in the blastula, the mes-ectoderm, primary entoderm and secondary entoderm. These germinal regions do not seem to have been previously recorded in Turbellaria.

4. The vitellarial yolk-cell membranes and nuclei degenerate after deposition in the capsule; no temporary epithelium is formed by them outside the yolk mass.

5. The primary entoderm cells which lie as a cap at the posterior end of the embryo, absorb a large part of the vitellarial yolk. When replete their nuclei immediately degenerate.

6. These cells are then grasped by the amoeboid secondary entoderm cells. After gastrulation the latter completely engulf and digest the former.

7. During gastrulation the ectoderm cells absorb the free vitellarial yolk left over after the action of the primary entoderm.

8. The development of the eyes embraces two phases. First the accessory pigment cell arises as a large clear vesicle very early in the differentiation of the embryo, and becomes gradually filled with melanin granules which crowd the nucleus to one side. Secondly the essential organ, the sight cell, develops externally to the pigment cell, into an invagination of which it pushes, and sends a nerve fiber backward into the anterior horn of the brain.

9. The sub-dermal muscle fibers develop in this Rhabdocoele, as in Nemerteans, by the differentiation of mes-ectodermic cells from which long slender fibers grow outward beneath the external epithelium.

10. The intestine consists of large distinct cells whose free ends extend into the lumen. It arises after birth by the inter-

calation between the yolk-laden primary entoderm cells of new cells derived from the secondary entodermic elements which during gastrulation remain in the region just posterior to the oesophagus.

11. The approximate time of appearance was noted for the subdermal glands and those of the alimentary tract; they are not present in the young worm at the time it leaves the mother, but have already developed in free-living specimens which have reached a length of 0.30 mm.

12. The cephalic glands appear in the embryo while it still lies in the capsule. There are three single-celled glands which develop in the mesenchyme between and anterior to the eyes. By the time the young worm is ready to leave the mother these glands have each become connected with the exterior through a fine process which penetrates the anterior body covering.

13. During very early embryonic life the vitellogenous cells migrate backward from the region of the gonads—just posterior to the pharynx. Most of them become grouped in the posterior end of the body, but a number come to rest at various points beneath the ectoderm in the posterior two thirds of the body. The vitellaria develop first from the large mass of cells between the intestine and the body wall and push gradually forward till they effect a union with the posterior end of the antrum. Meanwhile the isolated cells divide to form groups which soon anastomose with the main portions of the glands.

The testis and ovary of each side of the body become differentiated from a single gonad, the former arising from the anterior and the latter from the posterior end. In a 0.30 mm. embryo the two glands have become separated. Meanwhile a large group of cells has accumulated in the mid-ventral line between intestine and body wall. The anterior portion of this mass proliferates cells which surround the testis with a flat external epithelium and also form the vasa deferentia. The seminal vesicle arises in the next posterior portion of the mass by a rearrangement of cells about a lumen. It is composed of two layers of epithelia. At the same time the atrium commune develops in a similar manner. At this stage the antrum

femininum and oviducts exist only potentially in the undifferentiated mass posterior to the atrium.

The shell glands develop below the antral mass before the lumen of the latter appears. At this stage the testis has become functional; one can easily distinguish spermatogonia at the anterior end, the primary and secondary spermatocytes, spermatids and mature spermatozoa. Many of the latter now appear in the thick-walled seminal vesicle. The genital pore does not open until later.

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PLATE 1

EXPLANATION OF FIGURES

1 Outline from life of P. gemellipara somewhat flattened under a cover slip. Several stages of embryonic development shown within capsules × 64.

2 Three animals natural size and five others × 5 in characteristic shapes and poses.

3 The entire animal viewed from the left side as a semi-transparent object. To show particularly the position of the reproductive organs and their relation to other organs. × 88.

4 Frontal section through the upper end of the antrum femininum, the ends of the vitellaria and the most mature oöcytes of each ovary. × 200.

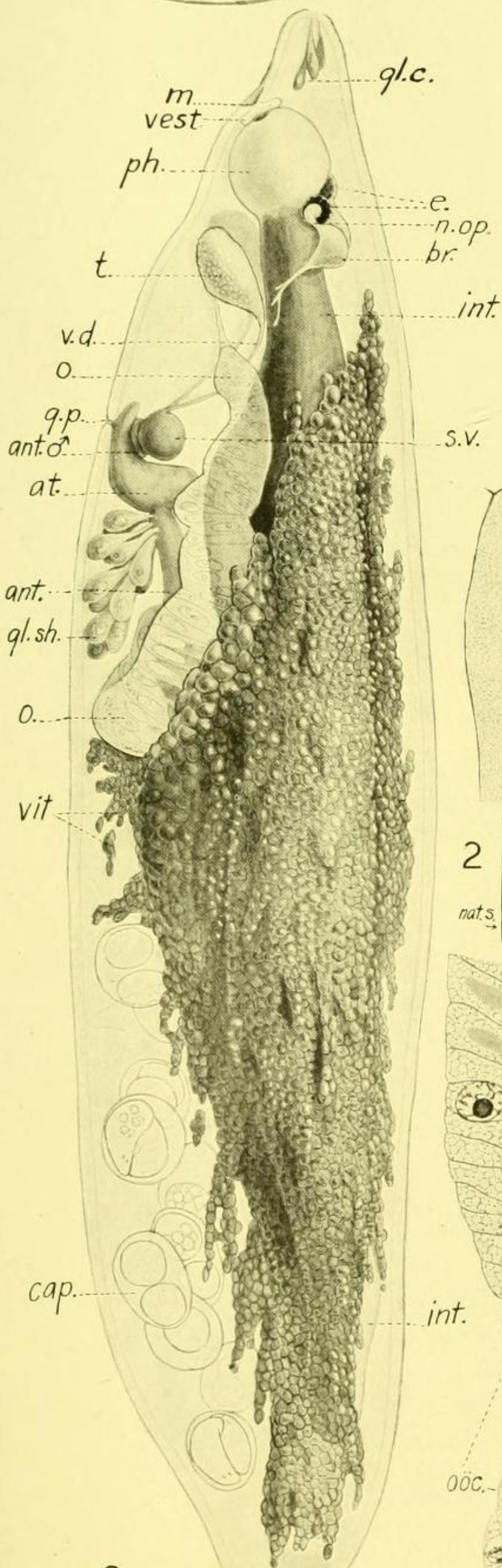
5 A spermatozoon. × 606.

6 Youngest third of the ovary to show the oögonia, development of the nucleolus, and particularly the development of the mitochondrial mass. × 606.

7 Portion of an oöcyte to show the structure of the cytoplasm and mitochondrial mass. × 606.

ant.♂, antrum masculinum
ant., antrum femininum
at., atrium commune
br., brain
cap., capsule
cyt., cytoplasm
e., eye
gl.c., cephalic glands
gl.sh., shell glands
g.p., genital pore
int., intestine
m., mouth
mes., mesenchyme
mit., mitochondrial mass
nat.s., natural size
n.op., optic nerve
nuc., nucleus
nucl., nucleolus
o., ovary
ooc., oocyte
oog., oogonia
ph., pharynx musculature
s.v., vesiculum seminalis
t., testis
v.d., vas deferens
vest., vestibule
vit., vitellarium
y.c., yolk cell

5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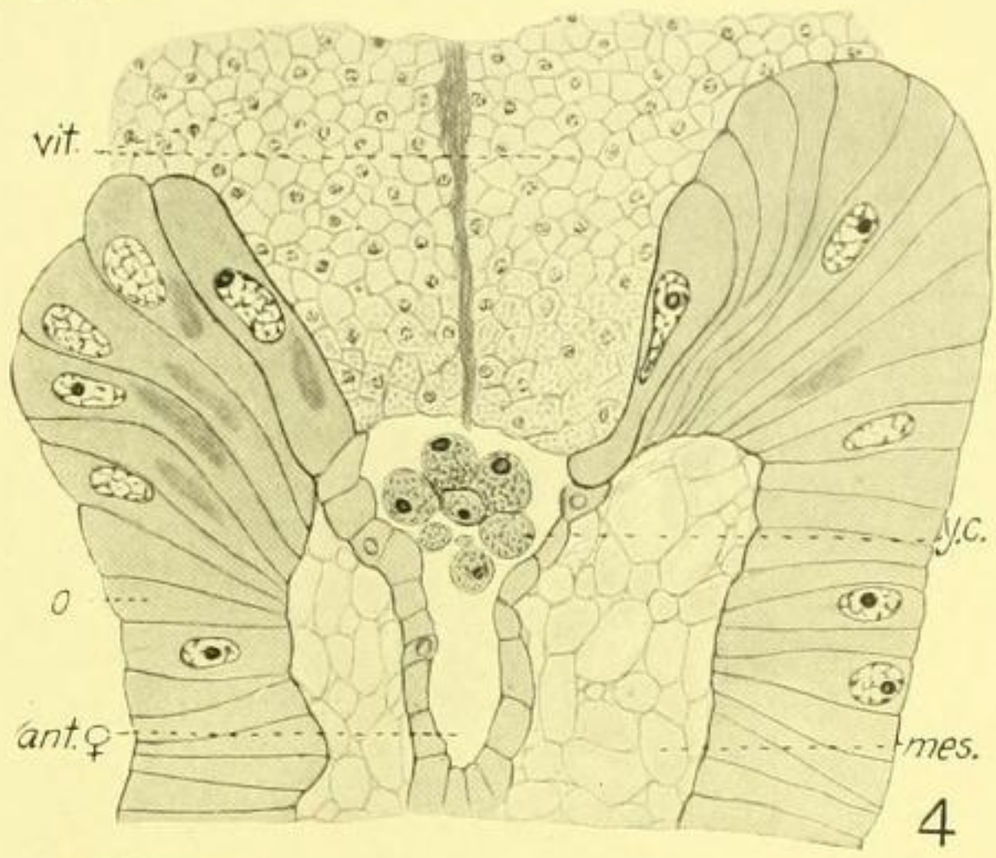
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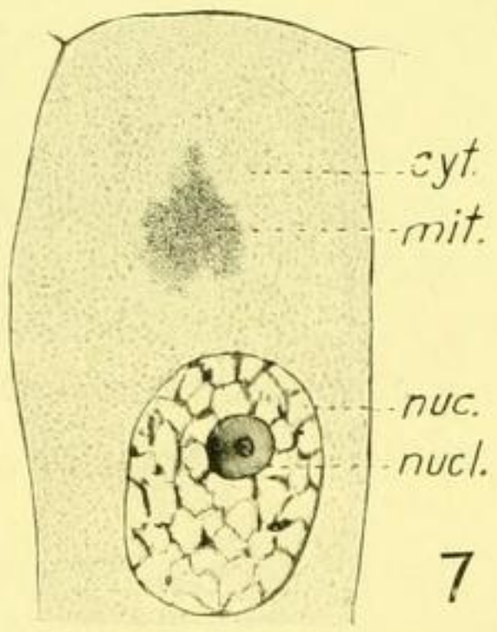
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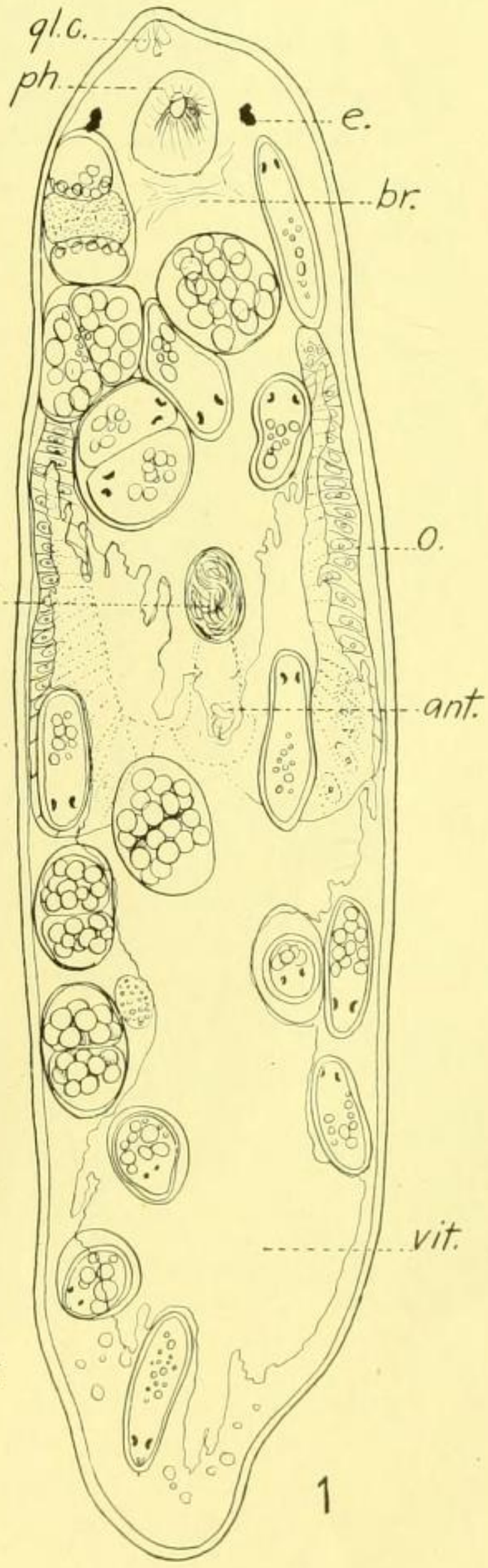
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PLATE 2

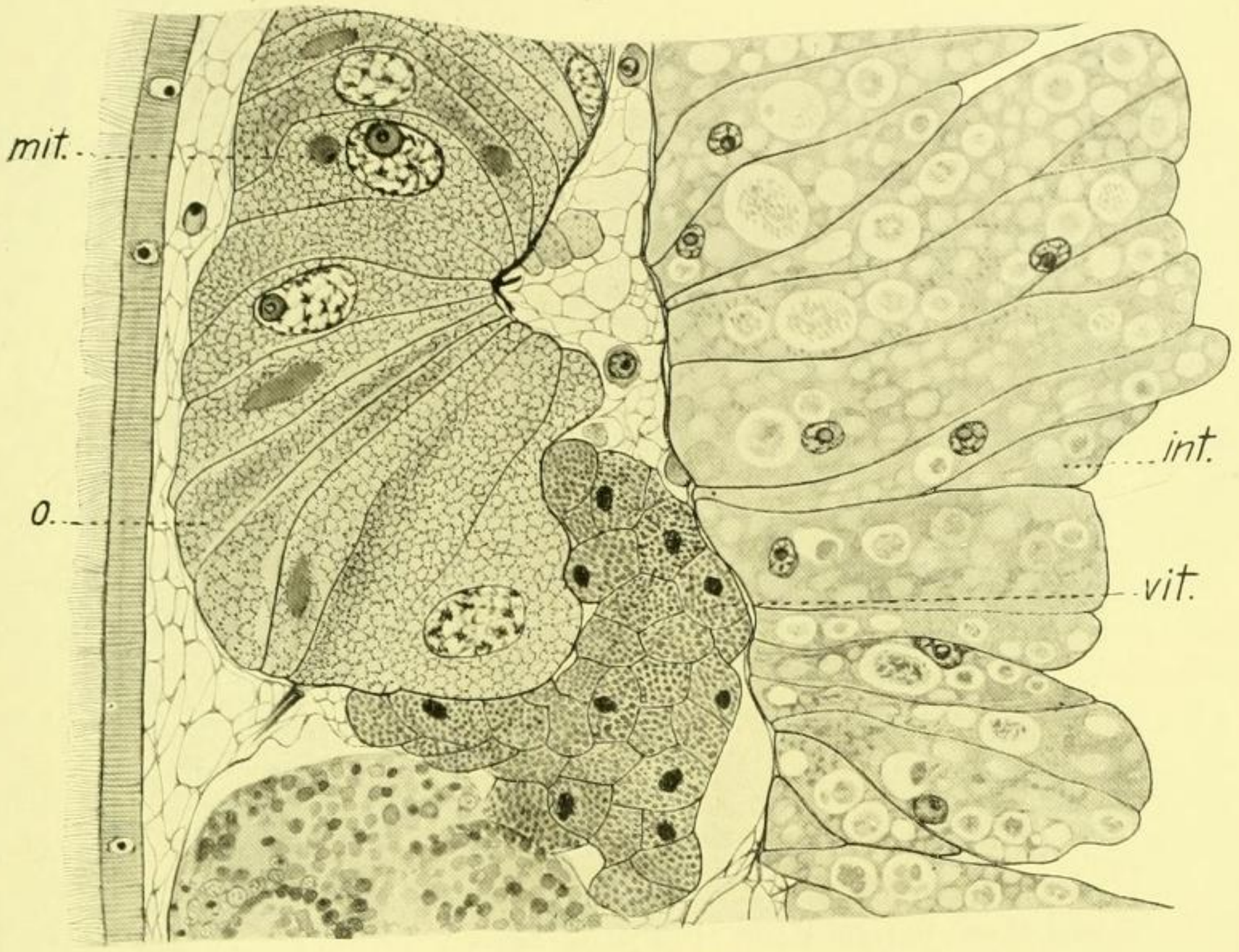
EXPLANATION OF FIGURES

8 Frontal section through the posterior end of the ovary as it curves inward toward the oviduct. Note the definite membranes about the ova. × 270.

9 Oblique sagittal section showing the crucial stage in the formation of the capsule. Two ova are being surrounded together with yolk cells by the secretion of the shell glands. Numerous spermatozoa present about the eggs, and a mass of them in the antrum. × 270.

ant., antrum femininum
at., atrium commune
int., intestine
mit., mitochondrial mass
o., ovary
sh.gl., shell glands
sh.sec., secretion of shell glands
sp., spermatozoon
s.v., vesiculum seminalis
vit., vitellarium
y., yolk

STANLEY C. BALL



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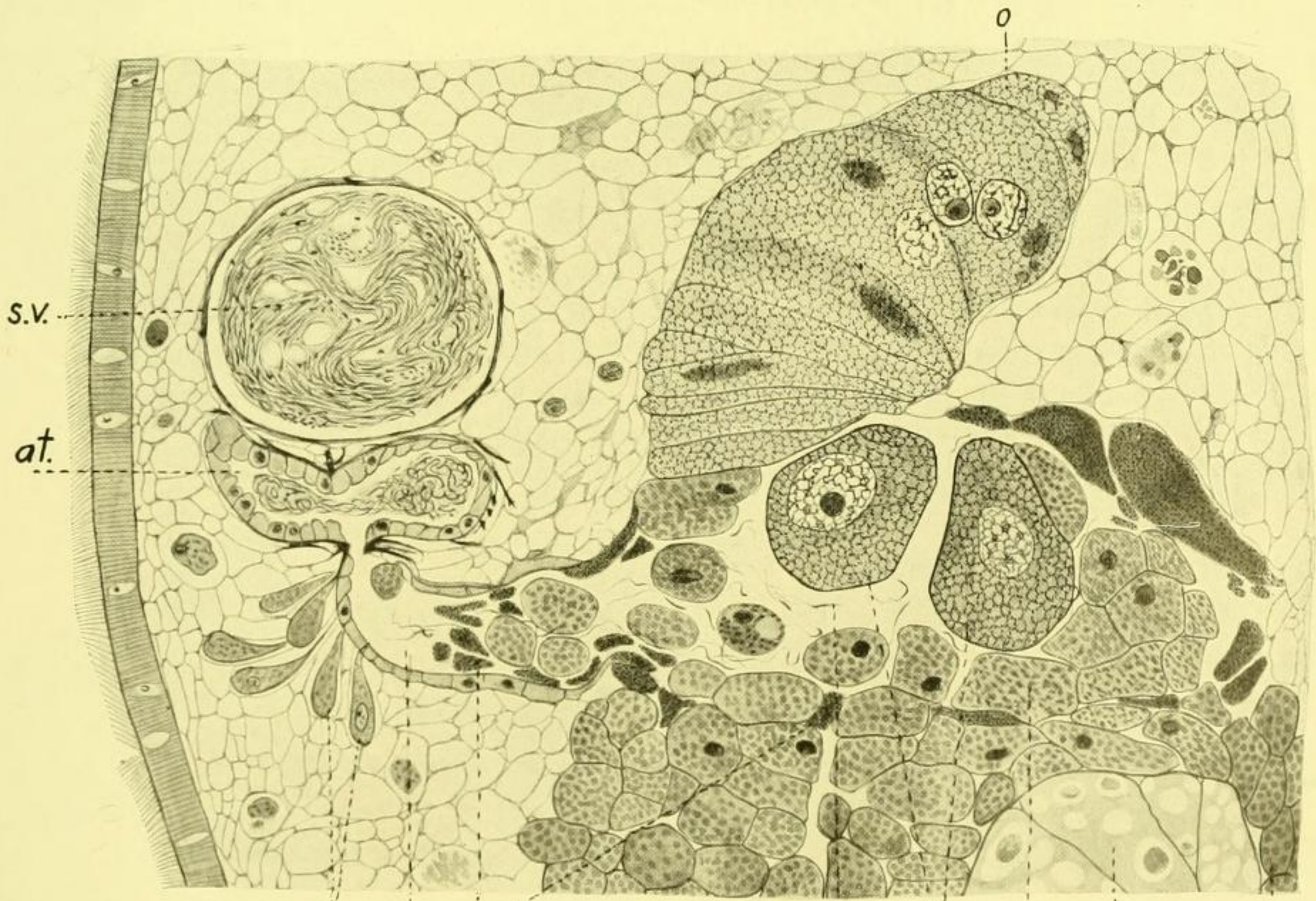

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PLATE 3

EXPLANATION OF FIGURES

10 Section of capsule showing first polar body at the left of the egg, and the male pronucleus inside. Several degenerating yolk cell nuclei and one degenerating spermatozoon in the yolk. × 730.

11 Another section of the same capsule in which the yolk cell membranes have not yet disintegrated. × 730.

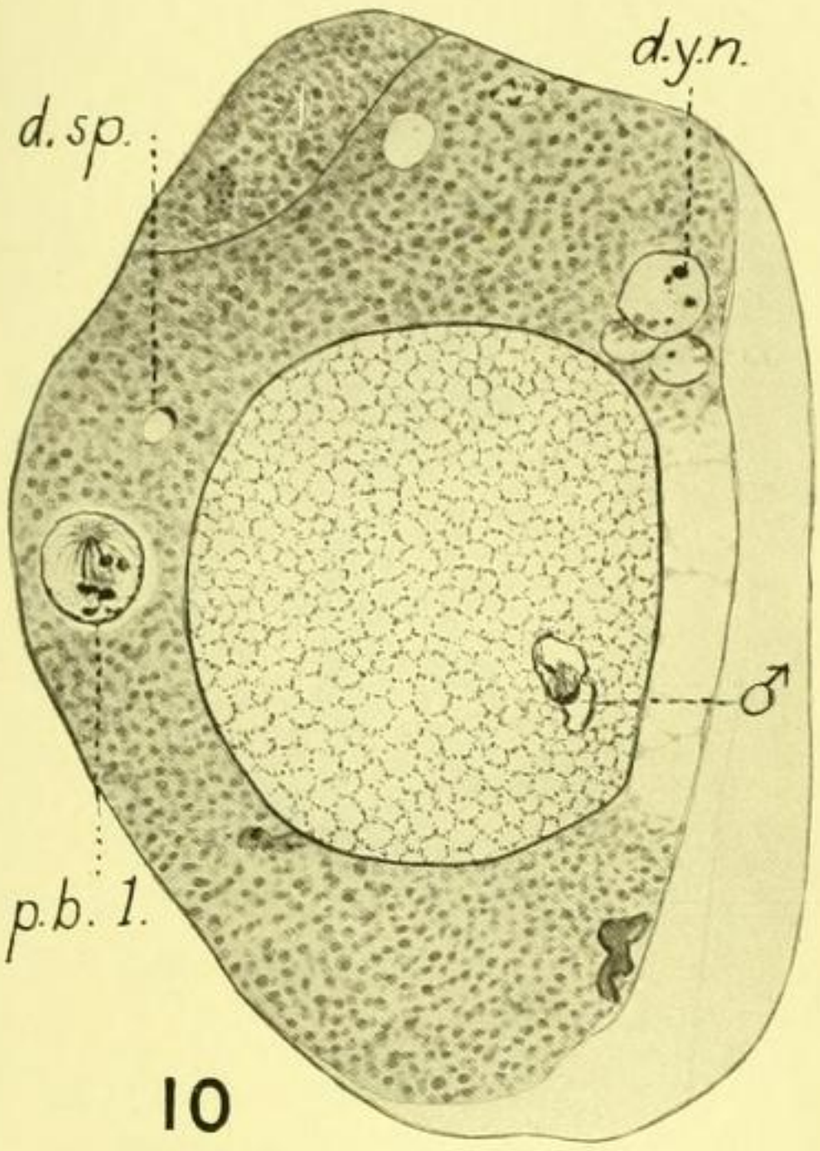
12 Drawing from life of a capsule freed from the mother by pressure, showing natural condition of yolk and mitochondrial mass, one egg and one embryo. × 520.

13 Two eggs of one capsule showing progressive steps in the cutting off of the first micromere. × 730.

14 Section through an embryo of about 72 cells. Three germinal regions are indicated, the primary entoderm (4 cells showing) at the left, mes-ectoderm at the right and secondary entoderm lying between. Yolk cell membranes and nuclei have entirely disintegrated. × 730.

15 Drawing from life of an embryo at the same stage as that in the preceding figure. × 730.

cap., capsule
cent., centrosome
d.sp., degenerating sperm
d.y.n., degenerating yolk cell nucleus
ent.1, primary entoderm
ent.2, secondary entoderm
inf., infiltration
mic.1, first micromere
mit., mitochondrial mass
p.b.1, first polar body
y., yolk
y.c., yolk cell
♂, male pronucleus



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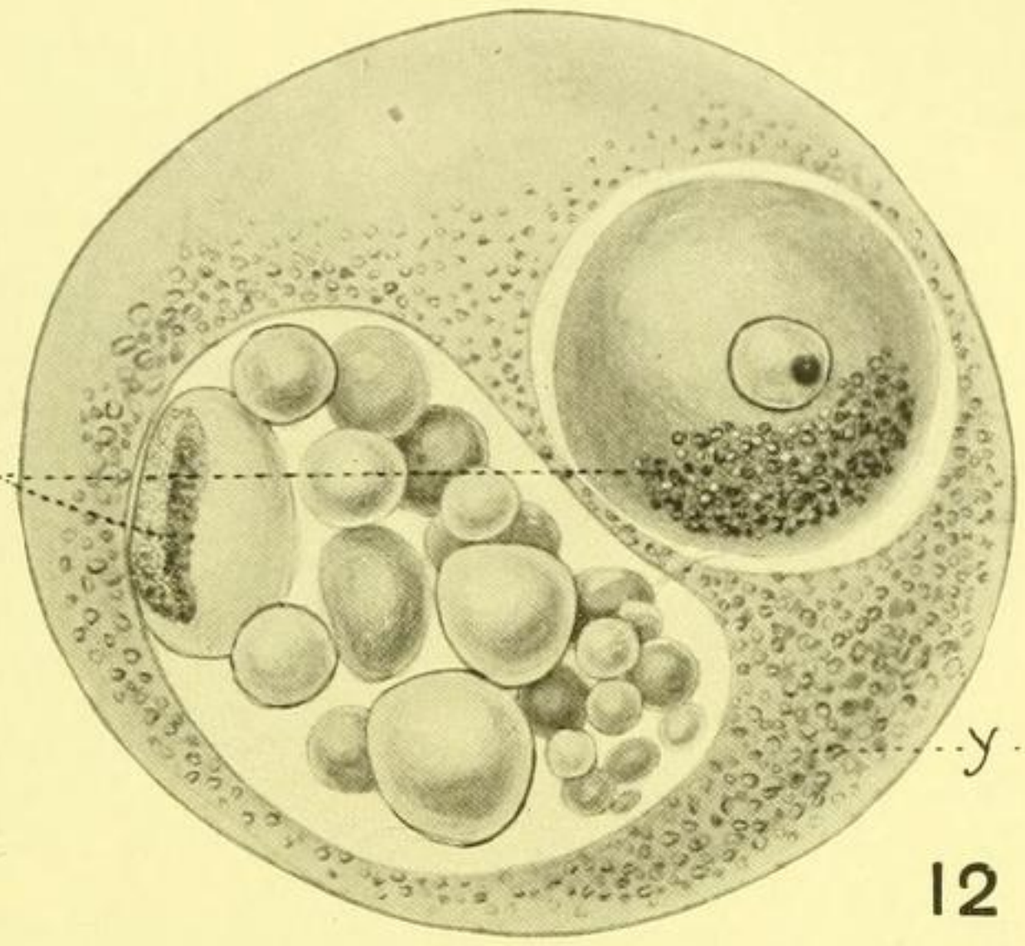

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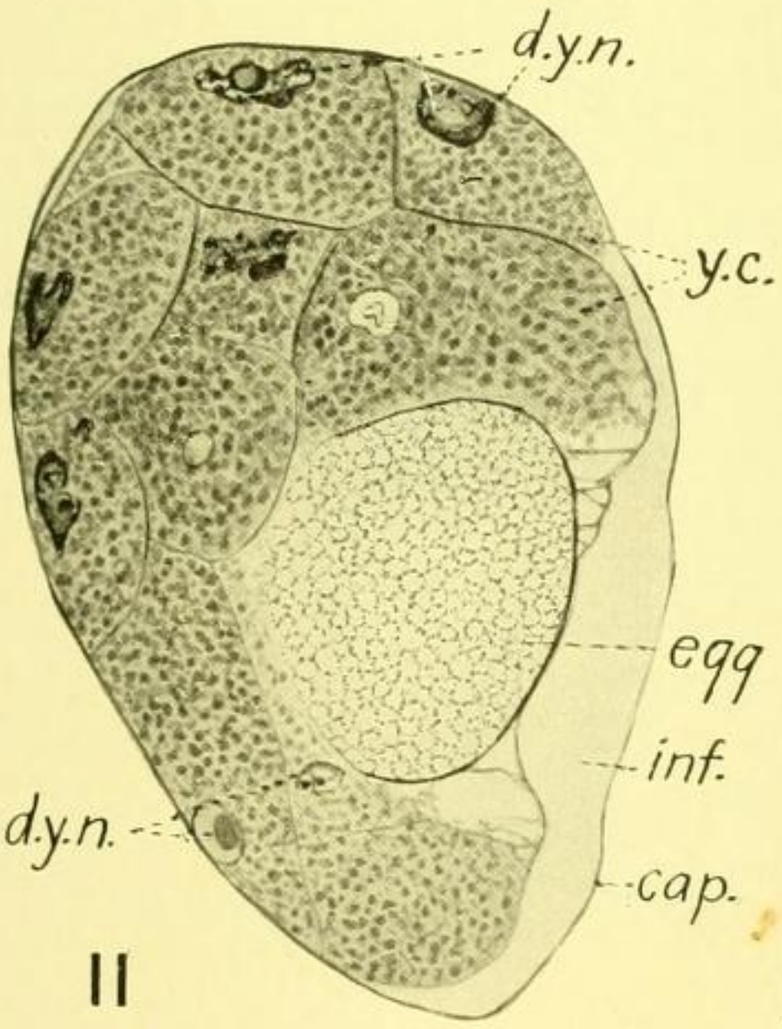

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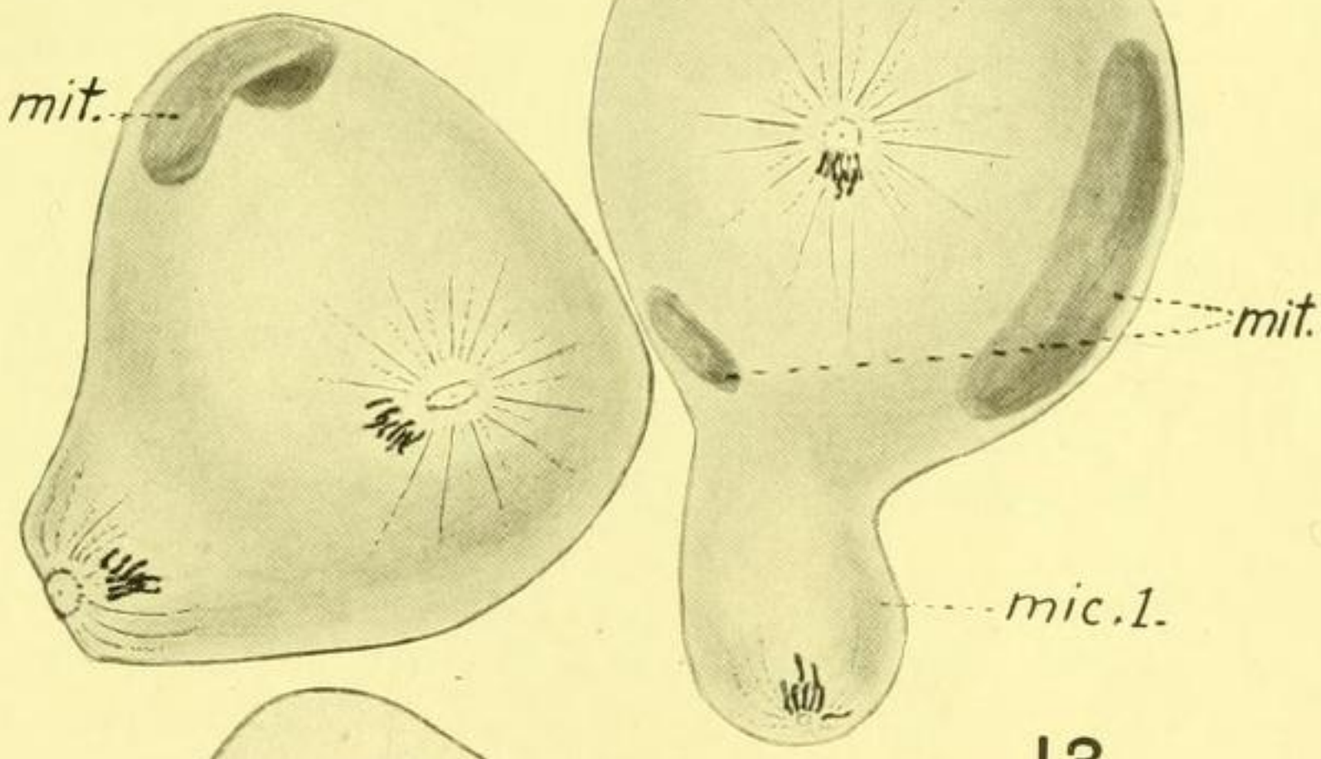

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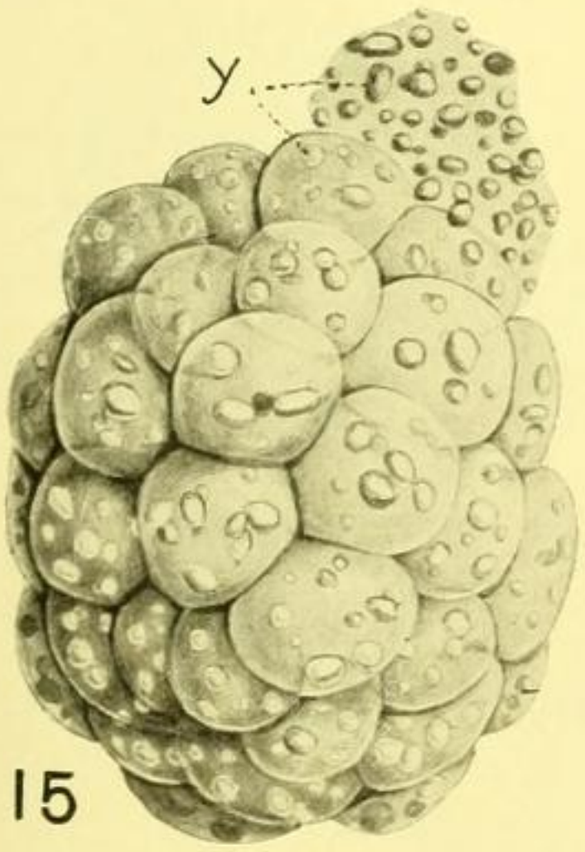

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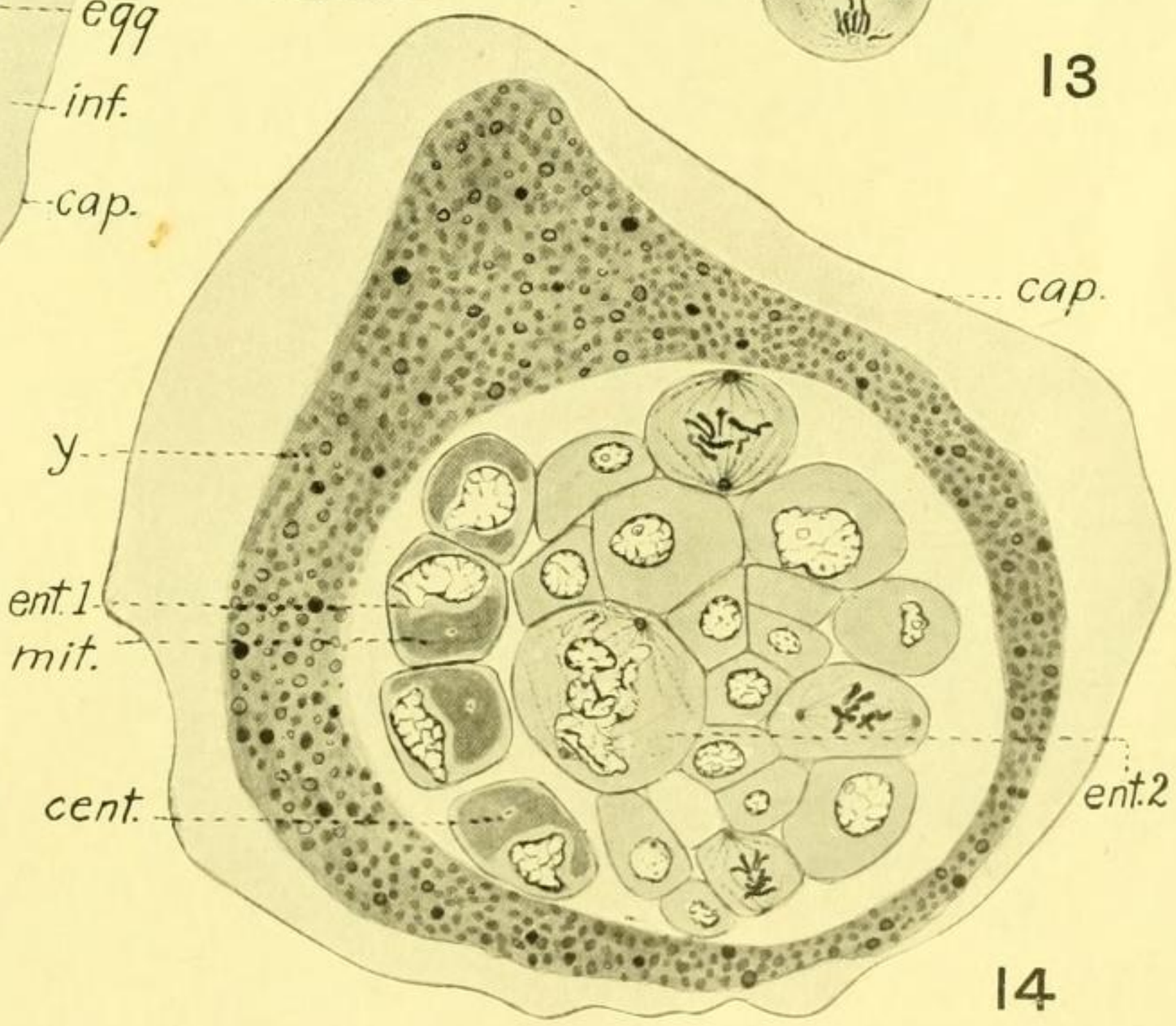

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PLATE 4

EXPLANATION OF FIGURES

16 Section through an embryo to show clearly the three germinal regions, mes-ectoderm, primary and secondary entoderm. × 730.

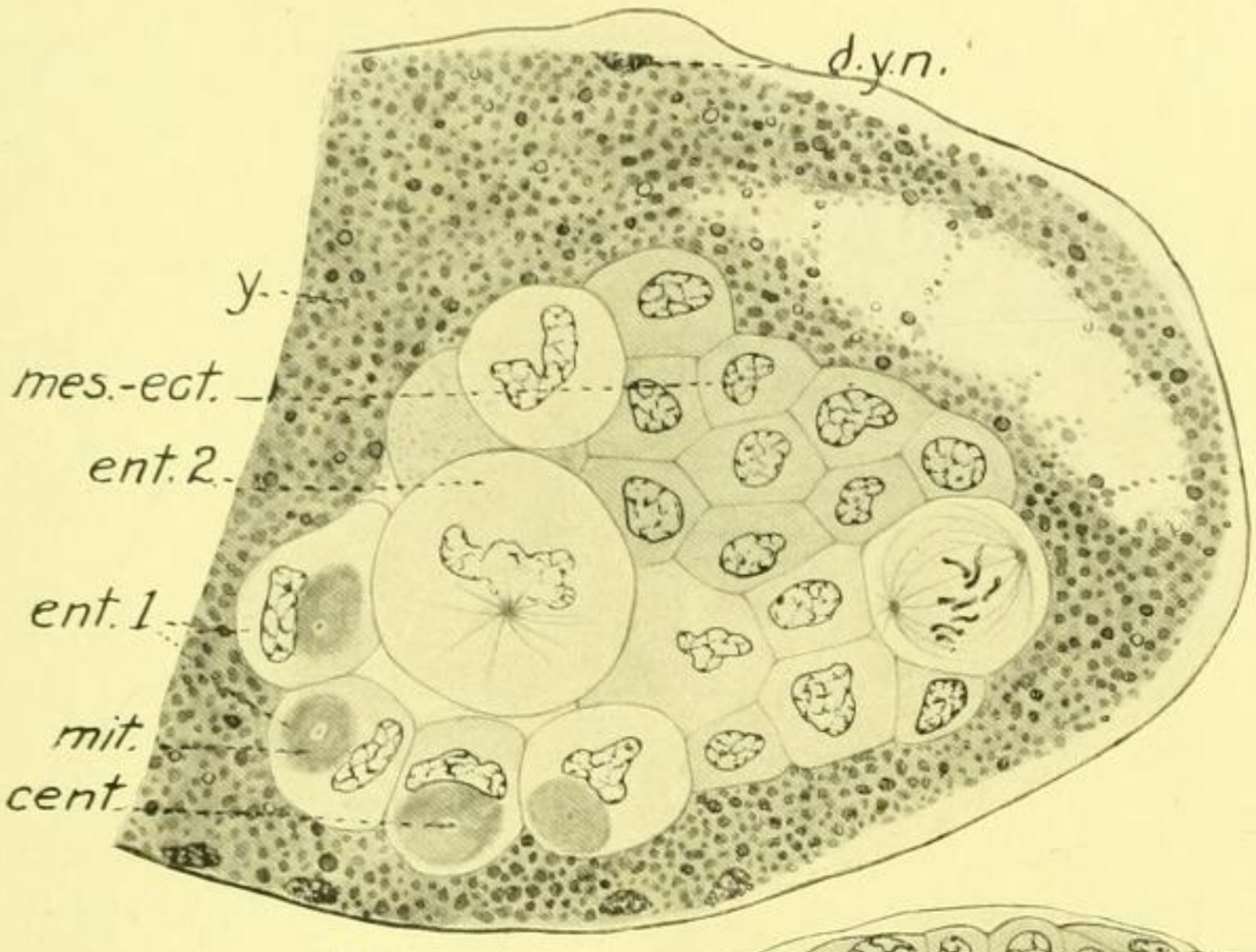
17 Section through a capsule of which the two embryos have begun to absorb yolk. This process is carried on by the secondary entoderm cells; each shows the mitochondrial mass and several yolk granules. × 730.

18 Section through part of a capsule in which the secondary entoderm cells have wandered to the posterior side of the primary entoderm. Most of the yolk has been absorbed by the entoderm. × 730.

19 Cell showing probable method of division of the mitochondrial mass. × 520.

20. Mitochondrial mass after its first division. Centrosome inside at the left, outside at the right. × 520.

cent., centrosome
d.y.n., degenerating yolk cell nucleus
ect., ectoderm
ent.1., primary entoderm
ent.2, secondary entoderm
mes-ect., mes-ectoderm
mit., mitochondrial mass
y., yolk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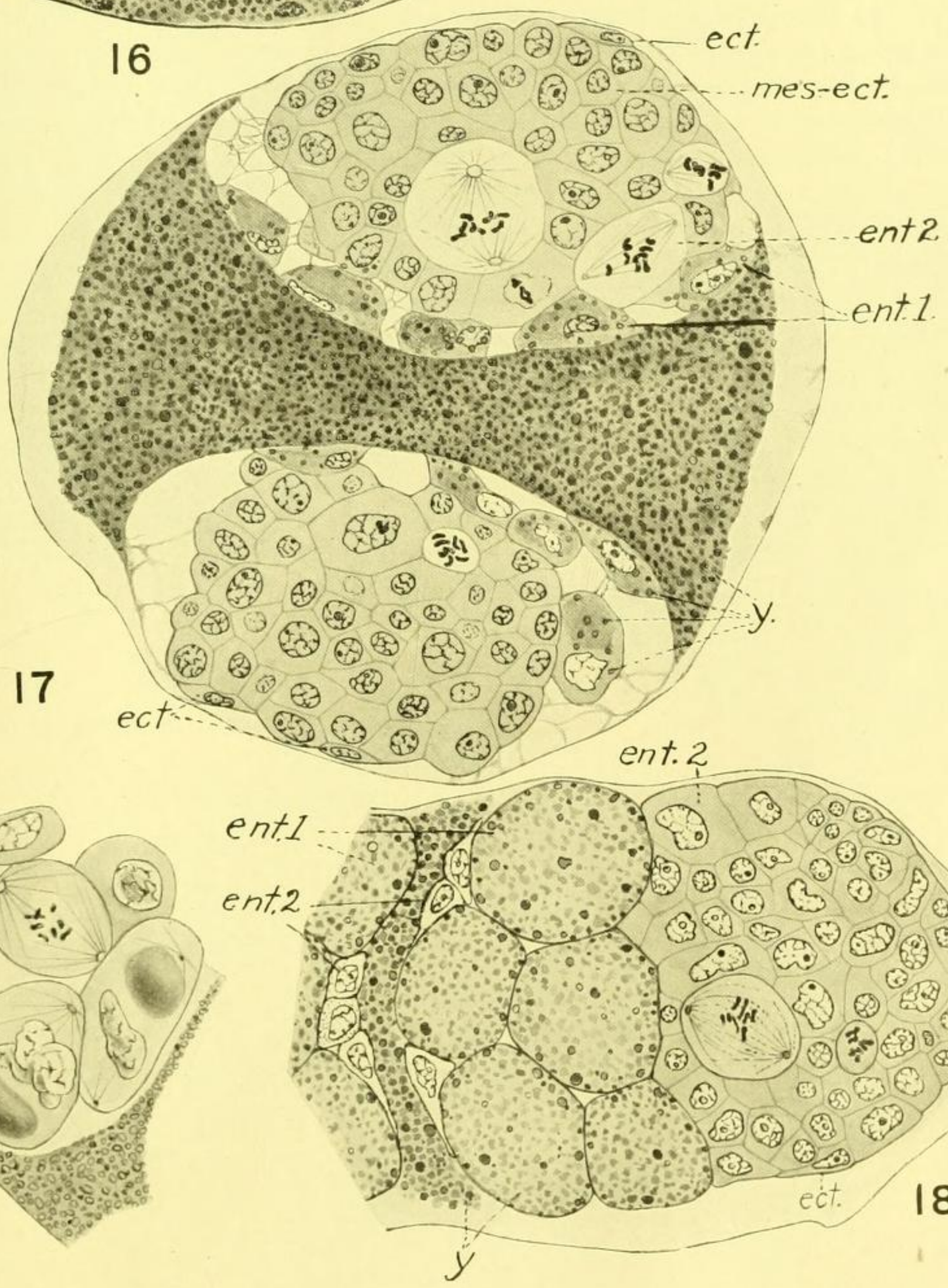
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PLATE 5

EXPLANATION OF FIGURES

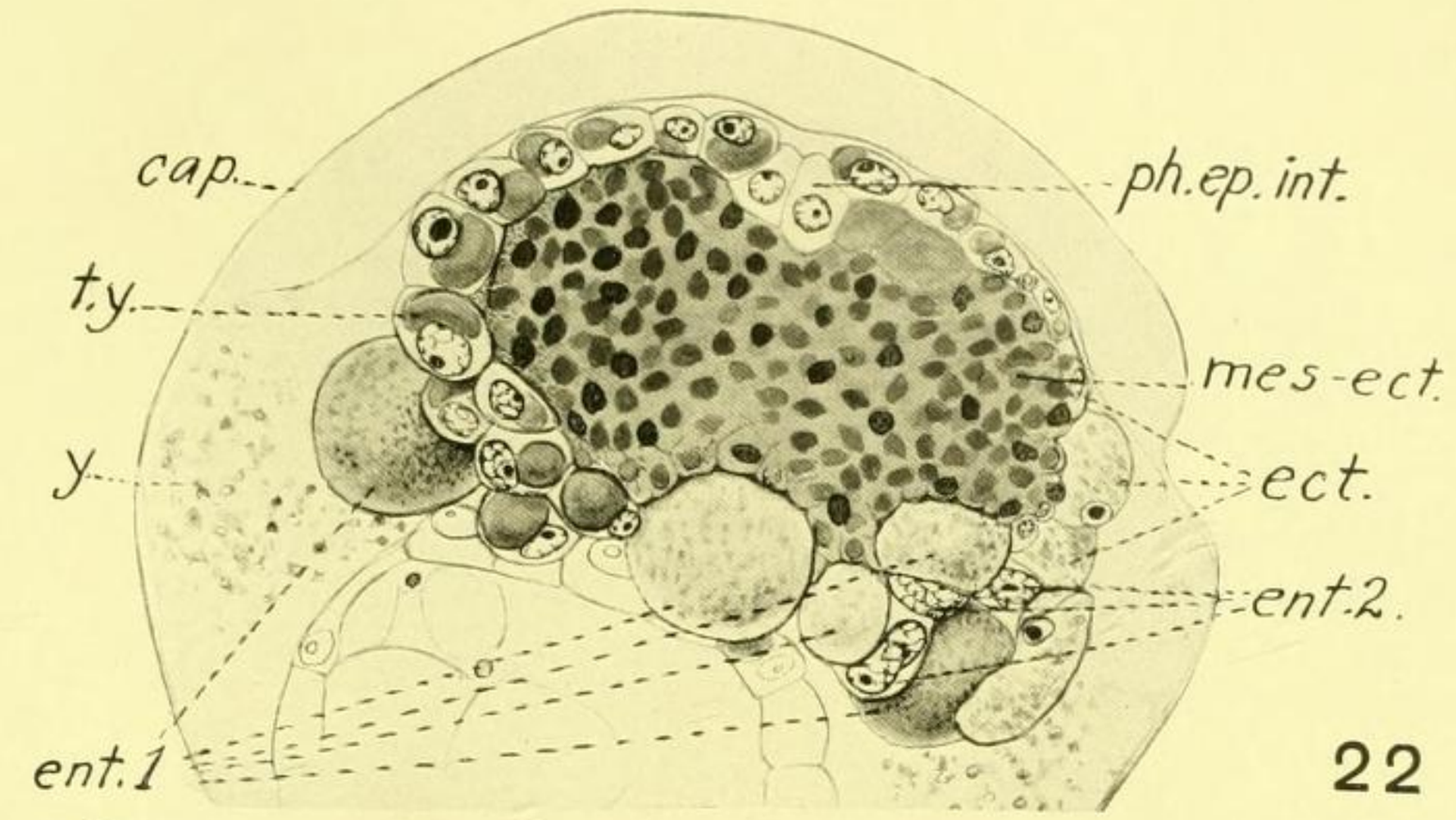
21 Section through two embryos of a capsule abundantly supplied with yolk. Nuclei of the primary entoderm cells are undergoing degeneration while these cells are being engulfed by those of the secondary entoderm. At *ect.* the mes-ectodermic cells are becoming flattened to form the ectoderm. × 730.

22 Thick section of capsule in which the upper embryo is in the process of gastrulation. The ectoderm cells have nearly closed over the entoderm. The primary entoderm cells have been grasped by those of the secondary entoderm. At the right three ectoderm cells are absorbing free yolk left in the capsule after the entoderm had become replete. Ectoderm at the left contains yolk which has already been partially transformed. × 730.

23 Oblique frontal section of an embryo just after gastrulation has been completed. Same features as in preceding figure. The ectodermic yolk beginning to be transformed (cf. the two cells at the top). The cells from which the intestine is mainly to be derived are shown (*ent. 2*) lying between the yolk-laden cells and the undifferentiated anterior cell mass. On the lower right several large nuclei with their cytoplasm are to be added to the ectoderm. × 730.

24 Oblique sagittal section of a slightly later stage in which the mesodermic cells have pushed backward between the posterior body wall and entoderm. Eye pigment cell just differentiated. × 730.

cap., capsule
ect., ectoderm
ent.1, primary entoderm
ent.2, secondary entoderm
e.p.c., eye pigment cell
mes., mesenchyme
mes-ect., mes-ectoderm
ph.ep.int., internal pharyngeal epithelium
t.y., transformed yolk
y., yolk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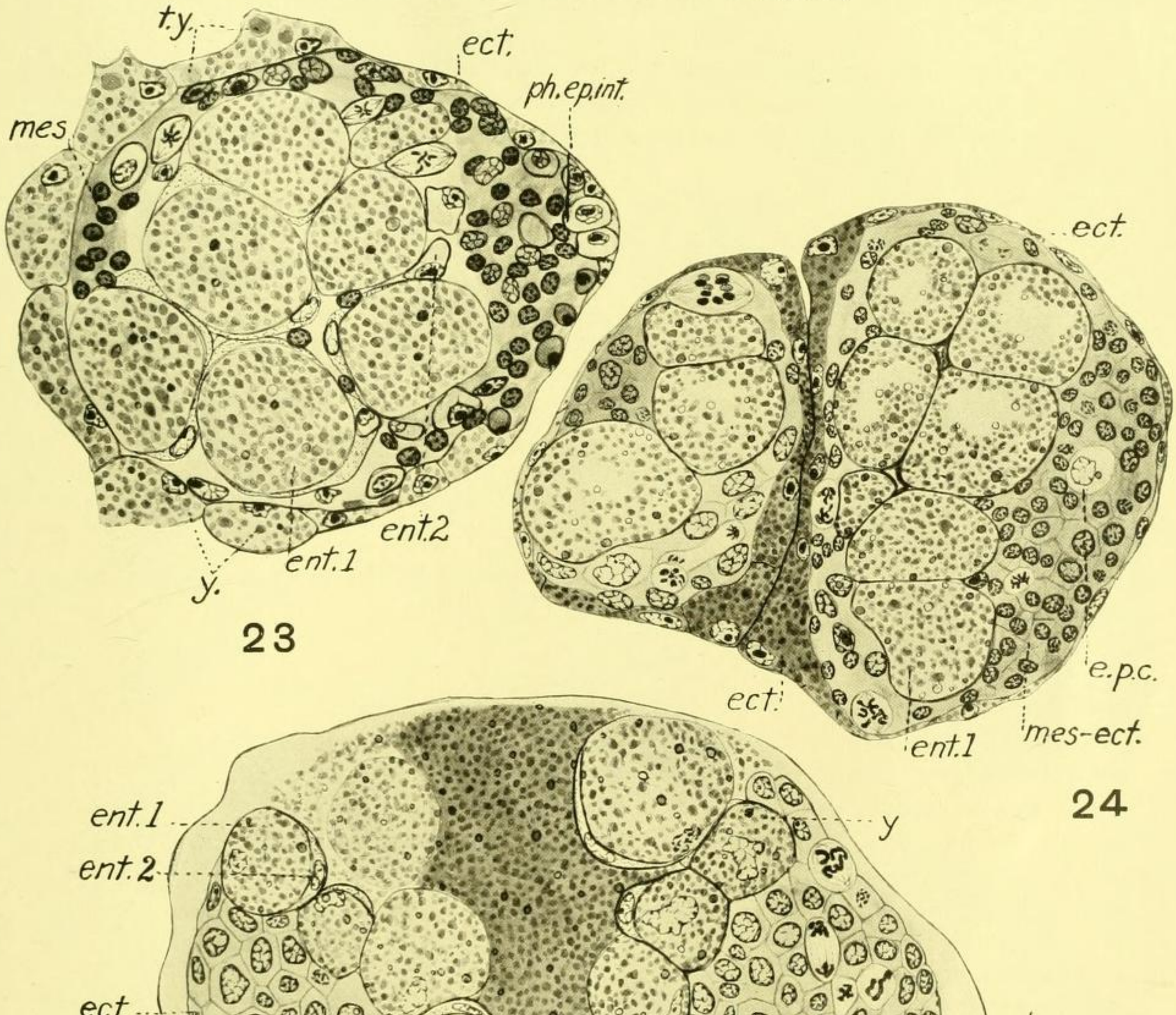
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mes-ect.

24

ent.1
ent.2
y
ect.
ect.

21

PLATE 6

EXPLANATION OF FIGURES

25 Drawing from life of an embryo at the stage seen in the preceding section (fig. 24). The globular nature of the free yolk is well shown. Water had infiltrated under observation between capsular membrane and yolk. Stained intra vitam with neutral red. × 730.

26 Frontal section of an embryo at same stage as that in preceding figure. Same features as in the latter and also the secondary entoderm cells (*ent. 2*). × 520.

27 Transverse section through pharynx and most anterior entoderm cells. Internal pharyngeal epithelium clearly illustrated in section, and the cells which are to form the pharynx musculature are grouped around this epithelial rod. × 520.

28 Frontal section of anterior end of an embryo to illustrate position of the two eye pigment cells. × 1016.

29 Oblique transverse section through brain and posterior nerve trunks. × 730.

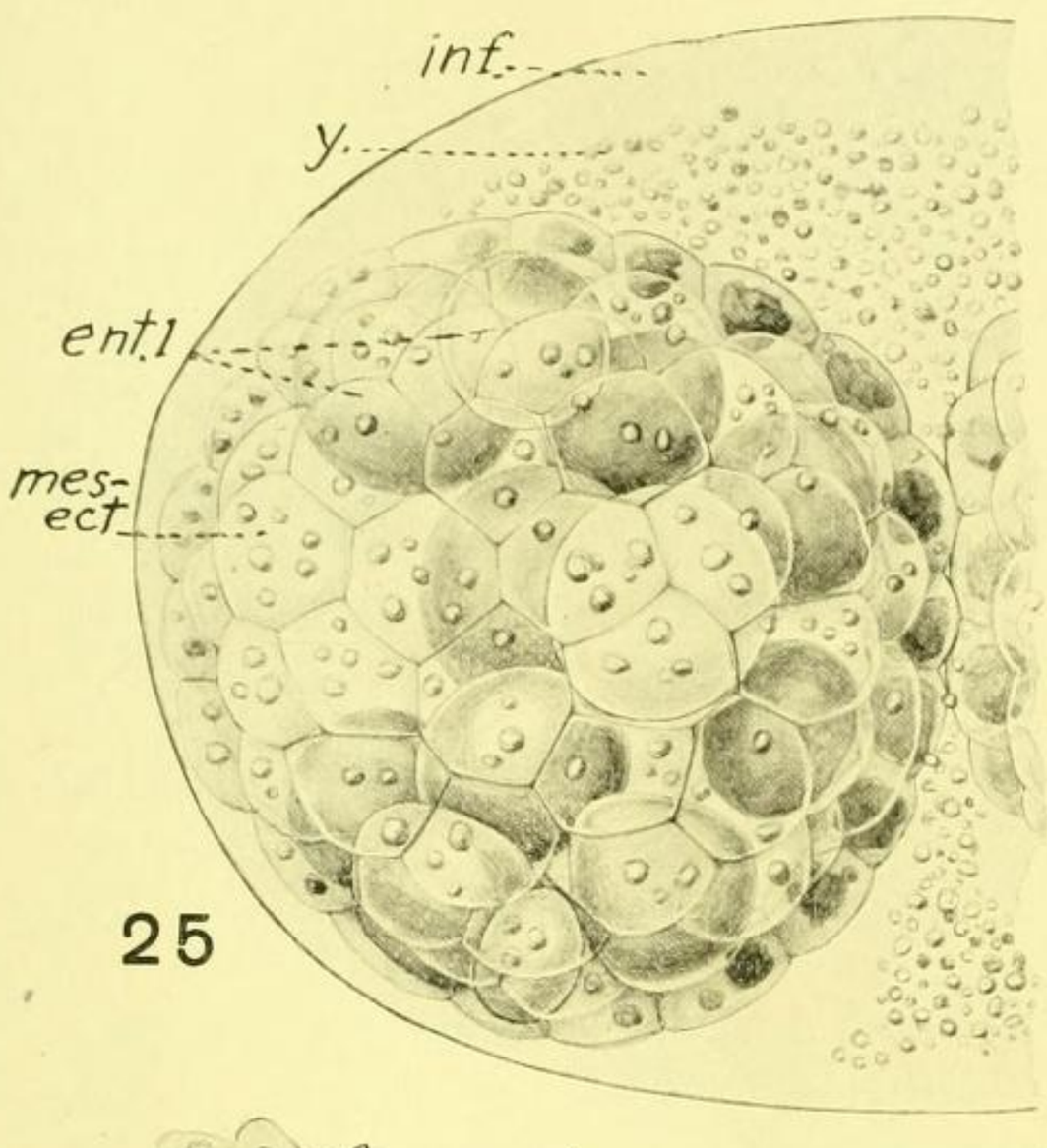
30 Series of drawings to show one type according to which the pigment granules of the eye appear one after another. × 530.

31 A second series to show another type of melanin deposition whereby several tiny spherules appear together and enlarge gradually. × 530.

32 Eye of an older embryo which had escaped from its capsule. Pigment cell has become invaginated by pressure of the ganglion cell (*g.c.*) from which nerve fibers extend into the brain. × 730.

33 Adult right eye seen from above showing the sight cell at the end of the optic nerve. × 730.

br., brain
ect., ectoderm
ent.1, primary entoderm
ent.2, secondary entoderm
e.p.c., eye pigment cell
esoph., esophagus
g.c., ganglion cells
inf., infiltration
m.emb., embryonic muscle cell
mes., mesenchyme
mes-ect., mes-ectoderm
n., nucleus
n.op., optic nerve
n.p., posterior nerve trunk
pct.s., punktsubstance
ph., pharynx musculature
ph.ep.int., internal pharyngeal epithelium
t.y., transformed yolk
vest.ep., epithelium of vestibule
y., yolk



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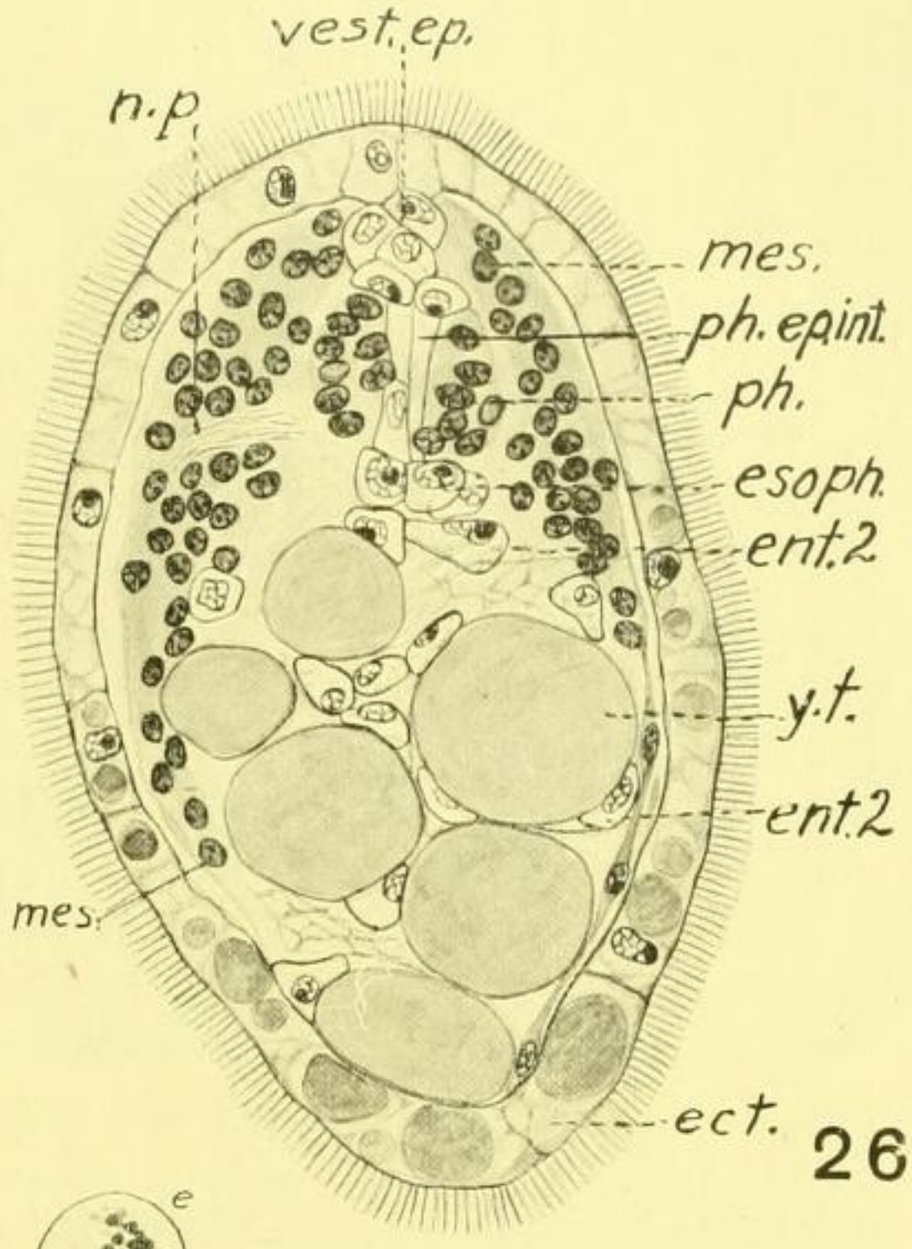

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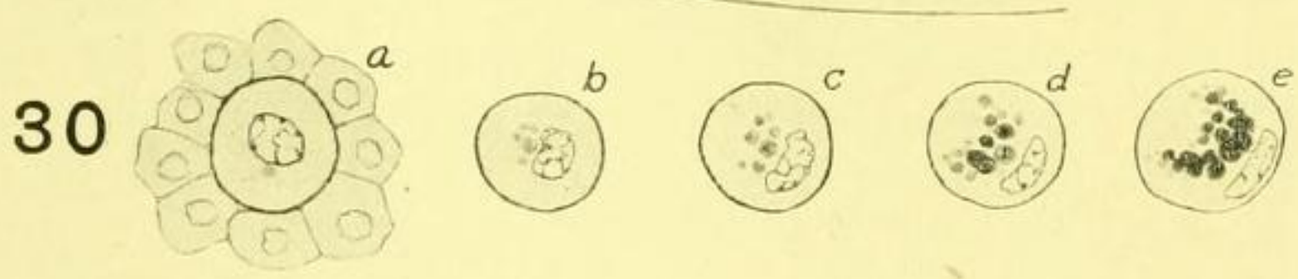

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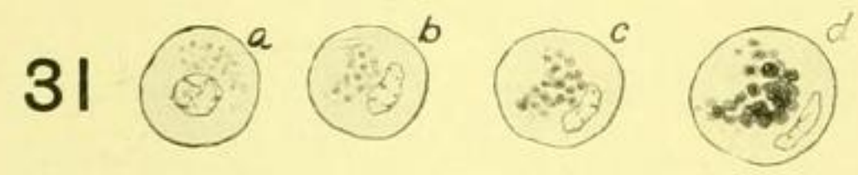

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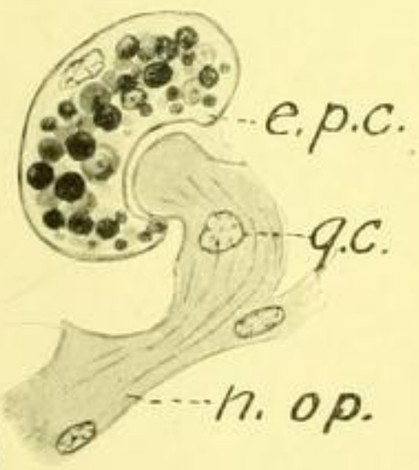

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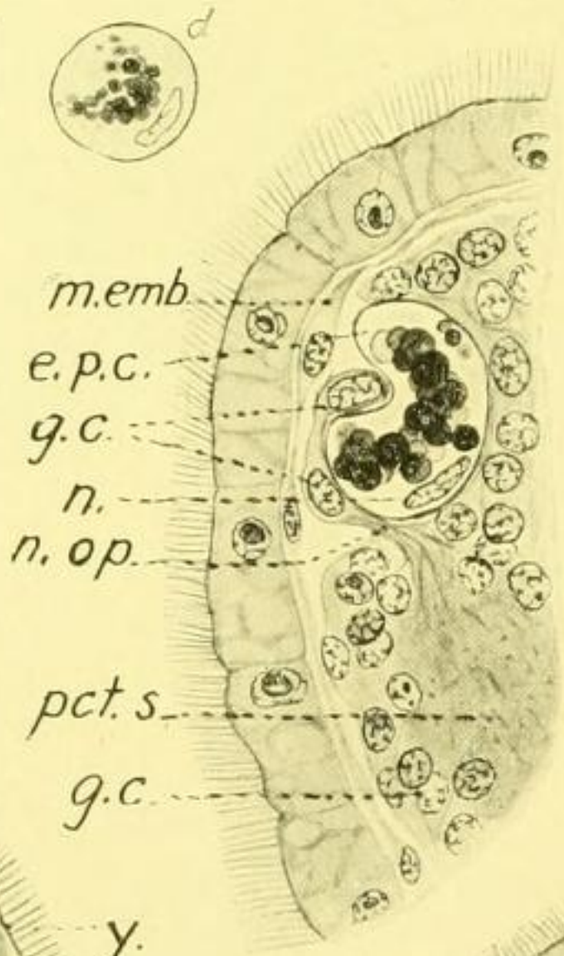

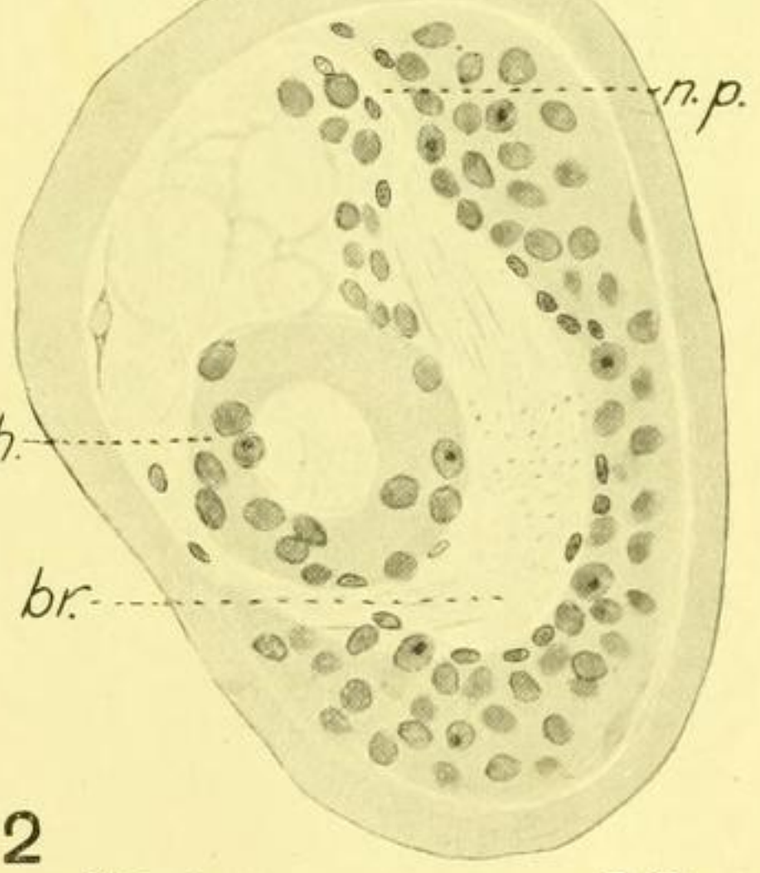

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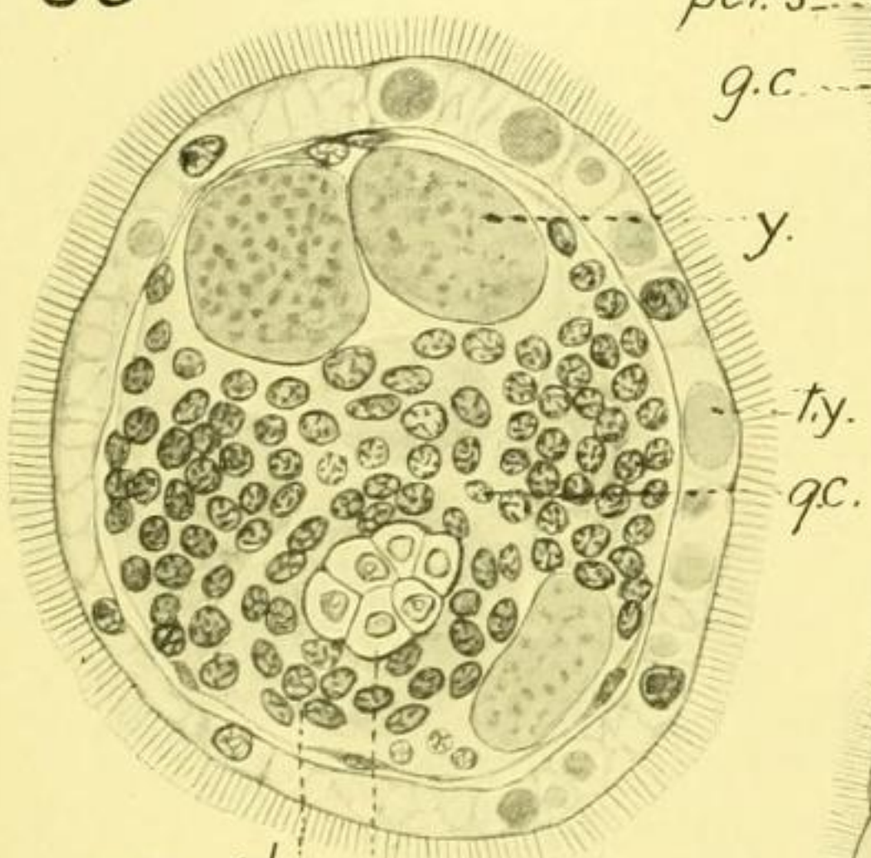

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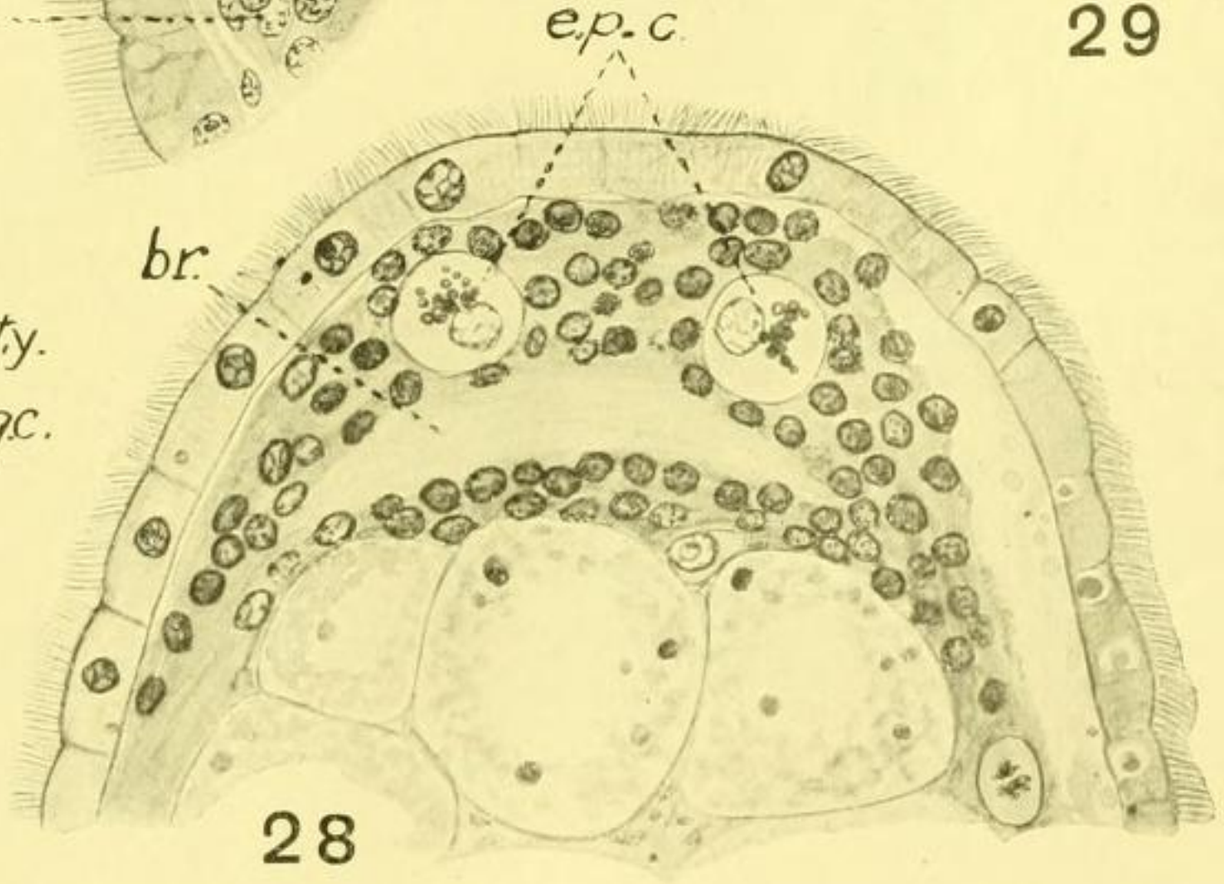

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PLATE 7

EXPLANATION OF FIGURES

34 Oblique sagittal section of an embryo somewhat more advanced than that in figure 33. Digestion of the yolk has begun; clear masses of transformed yolk occupy the center of each entoderm cell, while in the ectoderm the yolk has formed several irregular masses in each cell. The anterior ectoderm has become vacuolated. Between ectoderm and entoderm muscle cells are forming from mesodermic elements. Brain has differentiated and the internal epithelial rod has grown inward through the indistinct mass of pharyngeal muscle cells. Position of the mouth indicated at *m*. × 530.

35 Oblique sagittal section showing brain, eye pigment cell, entoderm and muscles. × 530.

36 Transverse section of same embryo as that shown in figure 27, but farther posteriorly, passing through the cells which later form the esophagus. × 530.

37 Oblique sagittal section of an older stage than that shown in figure 34. Entodermic yolk completely transformed (does not stain with iron haemotoxylin) while that in the ectoderm exists as large spheres. Pharynx distinct and early vitellogenous cells (*vit c.*) differentiated. Cilia have developed. × 730.

38 Transverse section of pharynx to show its musculature, and internal and external epithelia. × 730.

39 Tangential section through body wall of an embryo which had become freed into the mesenchyme of the mother. Muscle cell (*m. emb.*) from which extend fine muscle fibrils; the latter become the definitive sub-dermal fibers. × 1016.

40 Portion of a transverse section of the dorsal body wall showing outer circular and inner longitudinal sub-dermal muscle fibers. × 1016.

41 Adult sub-dermal muscle fibers. × 1016.

ect., ectoderm
ent.2, secondary entoderm
e.p.c., eye pigment cell
esoph., esophagus
g.c., ganglion cells
gon., gonad
m., mouth
m.emb., embryonic muscle cell
m.f.c., circular muscle fibers
m.f.l., longitudinal muscle fibers
pct.s., punktsubstance
ph., pharynx musculature
ph.ep.int., internal pharyngeal epithelium
ph.ep.ext., external pharyngeal epithelium
t.y., transformed yolk
vit.c., vitellogenous cells
y., yolk

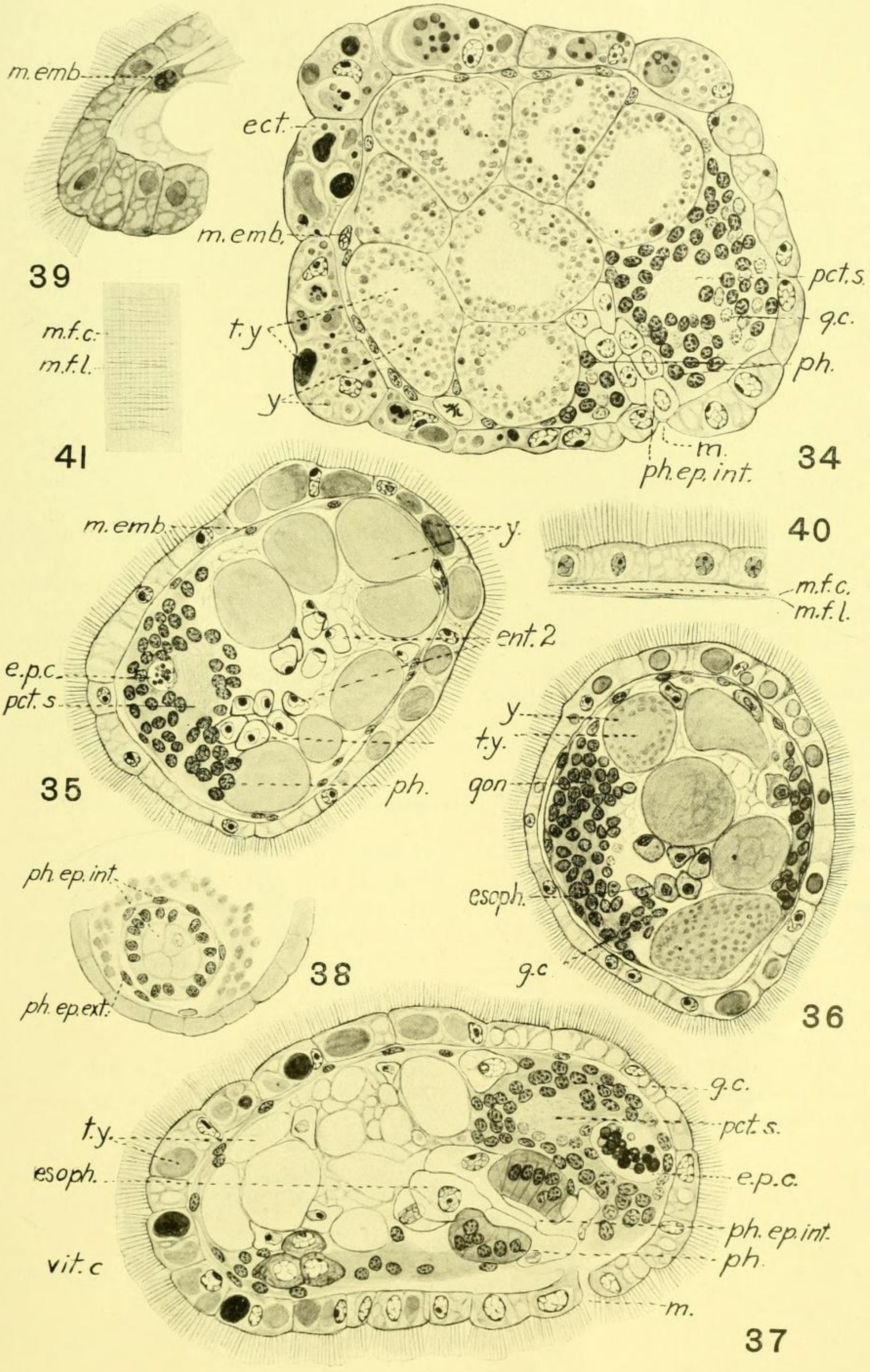

m.emb
ect.
m.emb.
pct.s.
g.c.
ph.
t.y
y
m.f.c.
m.f.l.
39
m.
ph.ep.int.
34
41
m.emb
y.
40
m.f.c.
m.f.l.
ent.2
e.p.c
pct.s
y
t.y.
gon
35
ph.
esoph.
ph.ep.int.
38
g.c
36
ph.ep.ext.
g.c.
pct.s.
t.y.
e.p.c.
esoph.
ph.ep.int.
ph.
vit.c
m.
37

PLATE 8

EXPLANATION OF FIGURES

42 Frontal section of an embryo somewhat younger than that in figure 37. The yolk masses are seen to be lying inside the secondary entoderm cells, having lost the membranes of the primary entoderm. Mitochondrial mass still visible with its centrosome. Yolk in the upper cell (*t.y.*) has been transformed to a fluid mass. × 730.

43 Sagittal section through an embryo which had not yet left the capsule. Drawn to show the vitellogenous cells migrating posteriorly. × 730.

44 Oblique transverse section through an embryo still in its capsule. Several large vitellogenous cells appear between the entoderm and sub-dermal muscle layer. Above are shown for comparison several cells at the tip of one of the mother's vitellarial strands. × 730.

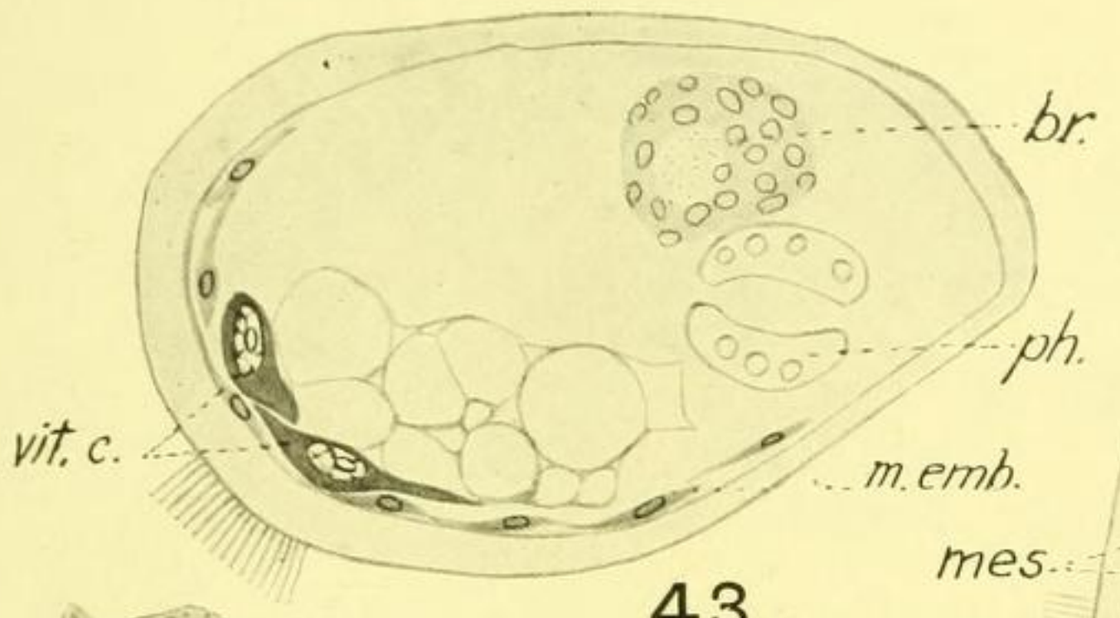
45 Anterior end of an embryo in which the cephalic glands are differentiating. Embryo still in capsule. × 730.

46 Cephalic glands at a later stage showing outlet to the exterior. Embryo free in the mother's mesenchyme. × 730.

47 Oblique frontal section of a young worm 0.30 mm. long found in sea water in which mussels had been washed. Relation of young testis and ovary is shown, as well as the fact that the vitellaria develop from the posterior mass of vitellogenous cells (figs. 43, 44). Cellular nature of the intestine is apparent. Subdermal and intestinal glands, and the structure of the mesenchyme are indicated. × 450.

48 Seminal vesicle of living adult containing several spermatids. × 808.

br., brain
e., eye
ent.1, primary entoderm
ent.2, secondary entoderm
gl.c., cephalic glands
gl.int., intestinal glands
gl.sub., subdermal glands
int., intestine
m.emb., embryonic muscle cell
mes., mesenchyme
mit., mitochondrial mass
o., ovary
ph., pharynx musculature
sp., spermatozoon
spt., spermatid
s.v., vesiculum seminalis
t., testis
vit., vitellarium
vit.c., vitellogenous cells
x., vitellogenous cells in mitosis
y., yolk



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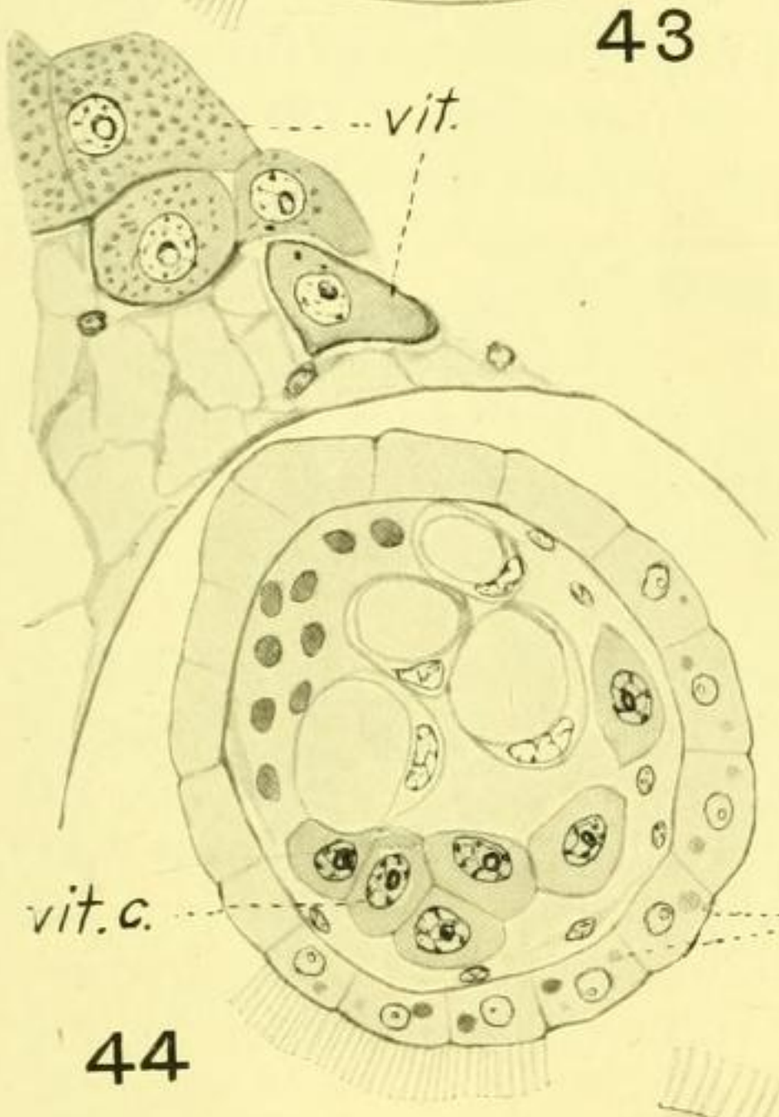

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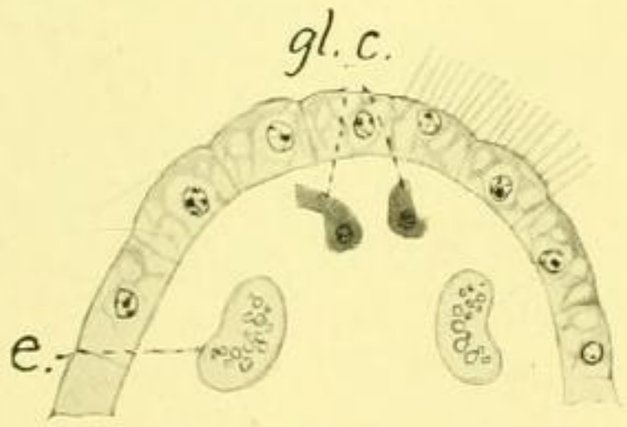

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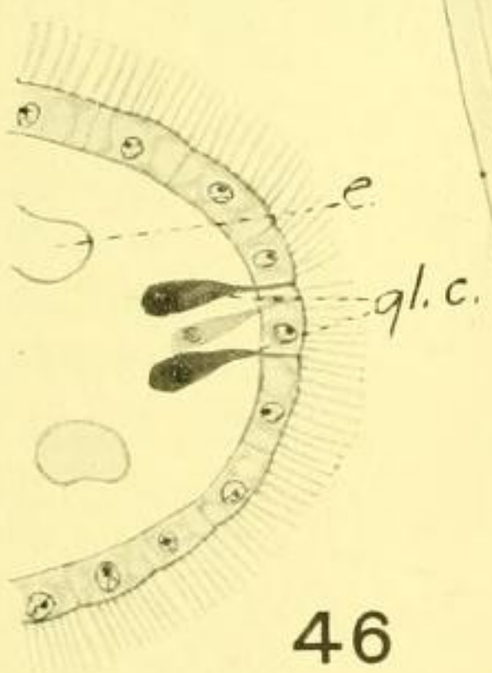

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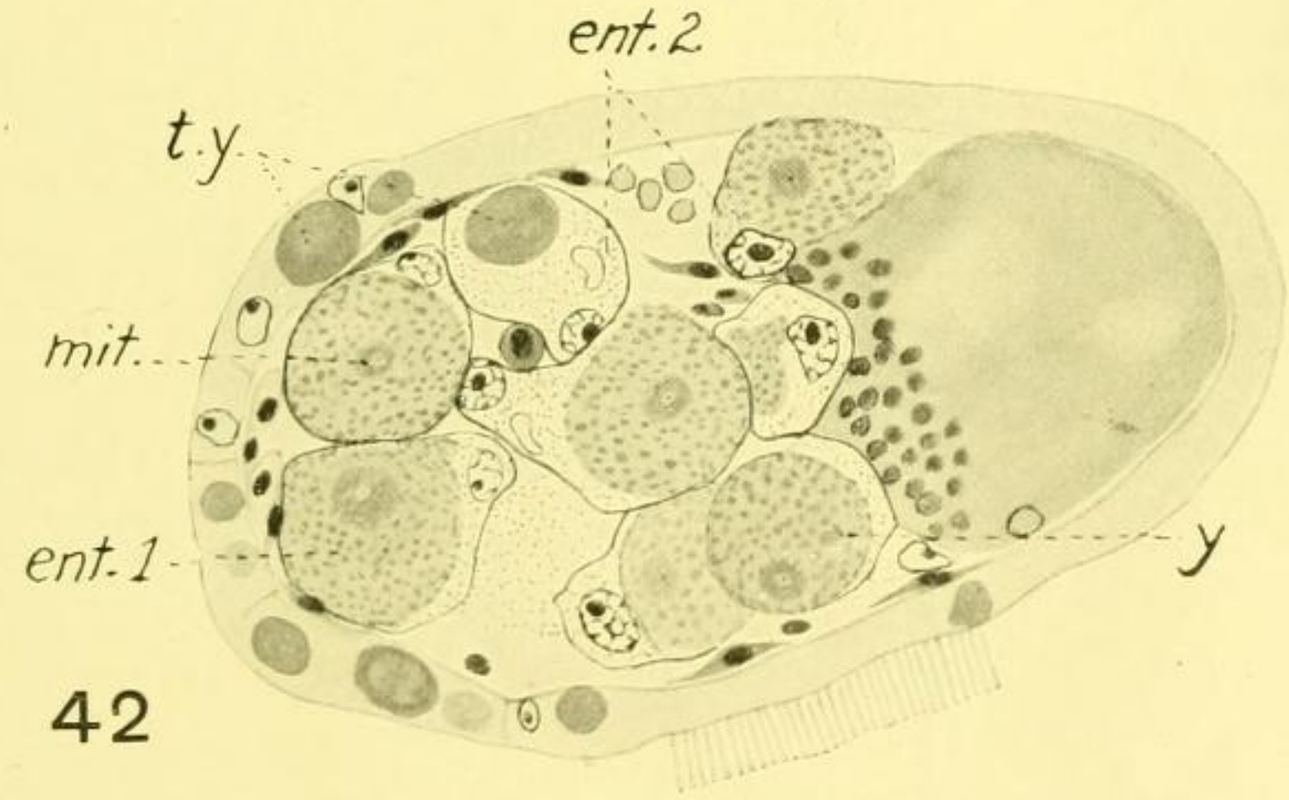

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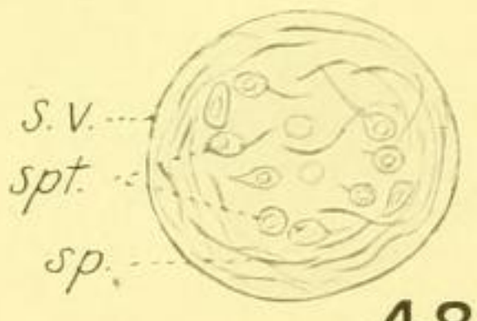

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PLATE 9

EXPLANATION OF FIGURES

49 Lateral view of anterior end of an embryo which had entered the mother's intestine. Drawn to show the gonads with cells in mitosis. × 730.

50 Sagittal section through the reproductive organs showing the undifferentiated cell mass which is to give rise to the atrium, antrum femininum and oviduct; also the shell glands (*gl.sh.*). × 400.

51 Sagittal section showing later stage in the development of the sexual organs. Testis has become functional showing spermatogonia, primary and secondary spermatocytes and mature spermatozoa. A number of the latter have already reached the seminal vesicle. Not yet has a lumen been formed in the antral region. × 400.

52 Reconstruction from sections of the reproductive organs of a 0.60 mm. worm which had been liberated from the mother for some time. The point of view is one looking posteriorly from a point just in front of the testes. × 270.

53 Oblique frontal section of an abnormal embryo in which the primary entoderm cells, having failed to absorb yolk according to their function, still remain in the center of the unusually small posterior half. The mesenchymatic cells have proliferated as in normal embryos and appear in several rows. × 530.

ant., antrum femininum
at., atrium commune
br., brain
ent.1, primary entoderm
ep.t., epithelium of testis
esoph., esophagus
gl.sh., shell glands
gon., gonad
g.p., genital pore
o., ovary
o.d., oviduct
ph., pharynx musculature
sp., spermatozoon
spc.1, primary spermatocyte
spc.2, secondary spermatocyte
spg., spermatogonia
s.v., vesiculum seminalis
t., testis
v.d., vas deferens
vit., vitellarium

STANLEY C. BALL