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S. M. Manton

Phil. Trans. R. Soc. Lond. B 1938 **228**, 421-441
doi: 10.1098/rstb.1938.0001

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STUDIES ON THE ONYCHOPHORA

IV—THE PASSAGE OF SPERMATOZOA INTO THE OVARY IN *PERIPATOPSIS* AND THE EARLY DEVELOPMENT OF THE OVA*

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(*Communicated by W. T. Calman, F.R.S.—Received* 15 *September* 1937)

[Plates 50 and 51]

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The Entry of Spermatozoa into the Female *Peripatopsis*

The males of some species of Onychophora form large spermatophores with a definite shape, and it is supposed that these spermatophores are deposited in the female genital opening. These species also possess paired receptacula seminis which contain most of the spermatozoa that may be found in the female genital tract. Near the receptaculum seminis there may be a ciliated funnel communicating with the body cavity (Peripatidae).

In other species, notably most of the Peripatopsidae, the male deposits small spermatophores anywhere on the body surface of the female. The receptacula seminis are almost or completely absent in *Opisthopatus* and *Peripatopsis*, and spermatozoa are found in the lumen of the ovarian tubes. No open communication exists between the female genital tract and the haemocoel.

The manner of entry of the spermatozoa into the female genital tract from the small spermatophores attached to the body surface has hitherto been unknown. As the

* Studies on the Onychophora. I, Digestive Enzymes of *Peripatopsis*, by N. G. Heatley, *J. Exp. Biol.* **13**, 329–43; II, The Feeding, Digestion, Excretion, and Food Storage of *Peripatopsis*, by S. M. Manton and N. G. Heatley, *Philos. Trans.* B, **227**, 411–64; III, The Control of Water loss in *Peripatopsis*, by S. M. Manton and J. A. Ramsay, *J. Exp. Biol.* **14**, 470–2.

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cuticle is dry, it is impossible for the spermatozoa to reach the external genital opening directly from the spermatophores, and the problem remains as to "how these little packets of spermatozoa get into the vagina, and then up the uteri, which are always full of embryos" (SEDGWICK 1885). WILLEY (1898) states that spermatozoa in *P. capensis* are "probably injected into the body-cavity through the body-wall by the process described by Whitman as *hypodermic injection*. In the case of leeches...this has been satisfactorily observed." This suggestion has been accepted as a fact by some later writers, and ZACHER (1933) remarks "Der Mechanismus der Begattung ist noch nicht aufgeklärt...bei *Peripatopsis capensis*...die Spermatophoren sollen die Körperwand durchdringen und in die Leibeshöhle gelangen", but no facts support these speculations.

SEDGWICK (1909) discusses the problem more fully. "It has been suggested that the spermatozoa make their way from the adherent spermatophore through the body wall into the body, and so by traversing the tissues reach the ovary; but having regard to the thickness and toughness of the skin and the absence of any cutaneous secretion capable of dissolving the coat of the spermatophore, it seems unlikely that this should occur. We therefore venture to make the suggestion, though we cannot offer any facts in support of it, except the swallowing of the cuticle..., that the creatures lick the spermatophores off their bodies or otherwise devour them, and that the spermatozoa are set free in the stomach, and make their way through its soft walls and through the body cavity to the ovary or receptaculum seminis."

Material for the solution of this problem has been provided by specimens of several species of *Peripatopsis* which have been kept in captivity during the last four years, and which have been killed at various seasons, primarily for other purposes (MANTON and HEATLEY 1937).

ZACHER's suggestion can be eliminated. Many spermatophores have been observed during the periods between ecdyses, and they are never detached from the body wall nor do they penetrate through it.

It is equally certain that spermatophores are not licked off the body by the female, but it is clear that spermatophores can reach the intestinal cavity when the cuticle is eaten after ecdysis. Numerous sections of intestines (prepared for other purposes) give no evidence of sperm gaining entry to the body cavity via the intestine. No sperm has been found in the intestinal lumen or in its walls; and it is probable that the protoplasmic contents, if any, of a spermatophore in the intestine are digested by the intestinal juices which are suited to a carnivorous diet (MANTON and HEATLEY 1937). Further, some spermatophores on cast cuticles are devoid of spermatozoa.

The occurrence of active spermatozoa, not only in the ovary, but in the haemocoel outside the egg follicles, was recorded by MOSELEY (1874). He remarks "they probably commonly escape amongst the viscera", and presumably he visualized the sperm escaping from the ovary to the haemocoel.

Details of the passage of the spermatozoa into the body have been followed in *P. sedgwicki*, but the process is probably similar in other species of the genus. Freshly

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deposited spermatophores have been watched until they are removed with the cuticle at ecdysis, and sections have been prepared of the body wall and attached spermatophore at known intervals after deposition. Duboscq Brazil proved to be a very satisfactory fixative, but embedding had to be done immediately after fixation in order to avoid hardening. Mallory's triple stain and iron haematoxylin were the most useful stains.

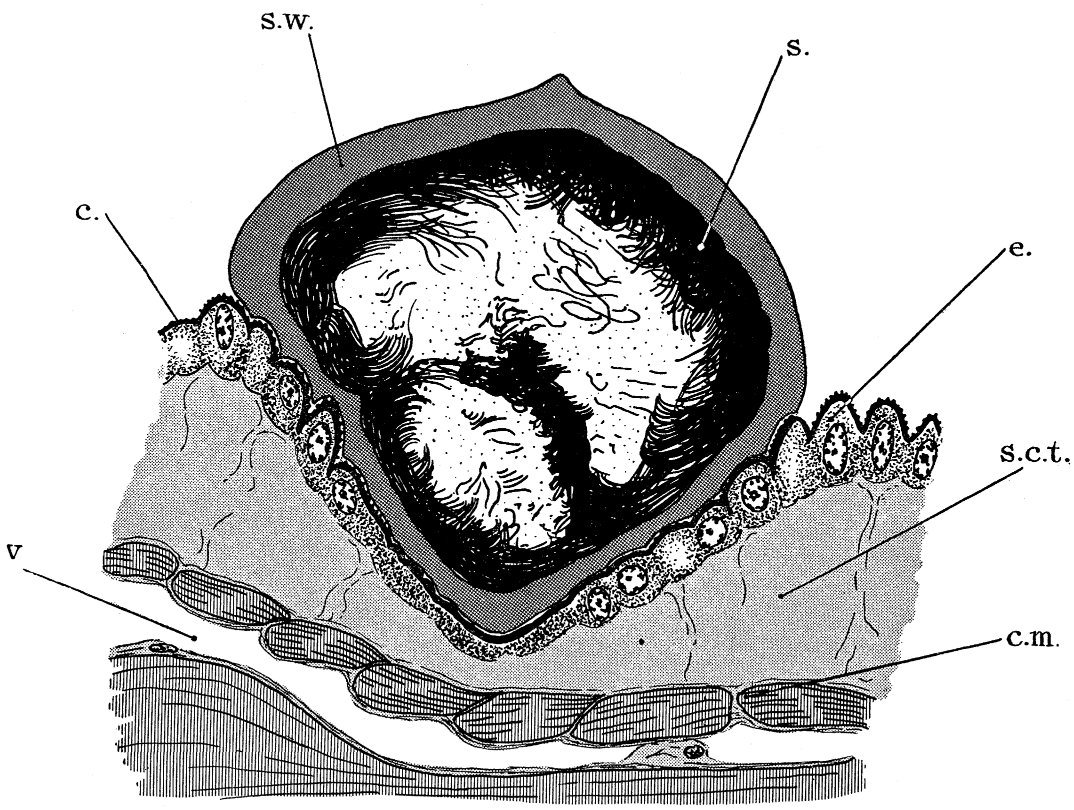

FIG. 1—Vertical section of a spermatophore attached to the body wall 48 hr. after deposition. The integument in this and the following figures is shown in black. (× 679 approx.)

The process of copulation in *Peripatopsis* will be described in a general account of the life-history which is in course of preparation. Each spermatophore is a closed capsule filled with sperm, and is placed anywhere on the body surface of the female. The spermatophores are variable in size and shape, they are usually irregularly rounded, and the diameter is often about 200μ. When freshly deposited they appear white and glistening, and adhere closely to the integument either between or over the primary papillae of the body. A small spermatophore fitting in between two papillae is shown in fig. 1. The wall of the spermatophore is tough and resistant and is evidently waterproof. It is at first fairly even in thickness, and in section appears to be composed of large granules stuck together. The wall stains red with Mallory's triple stain. Within it

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the spermatozoa are tightly packed. No change can be seen in either spermatophore or body wall for several days.

From the third to the fifth day after deposition of a spermatophore the thick subcutaneous connective tissue layer below it becomes invaded by cells which are either leucocytes from the haemocoel or cells derived from the intermuscular connective tissue. Normally very few cells can be seen in the outer connective tissue. These

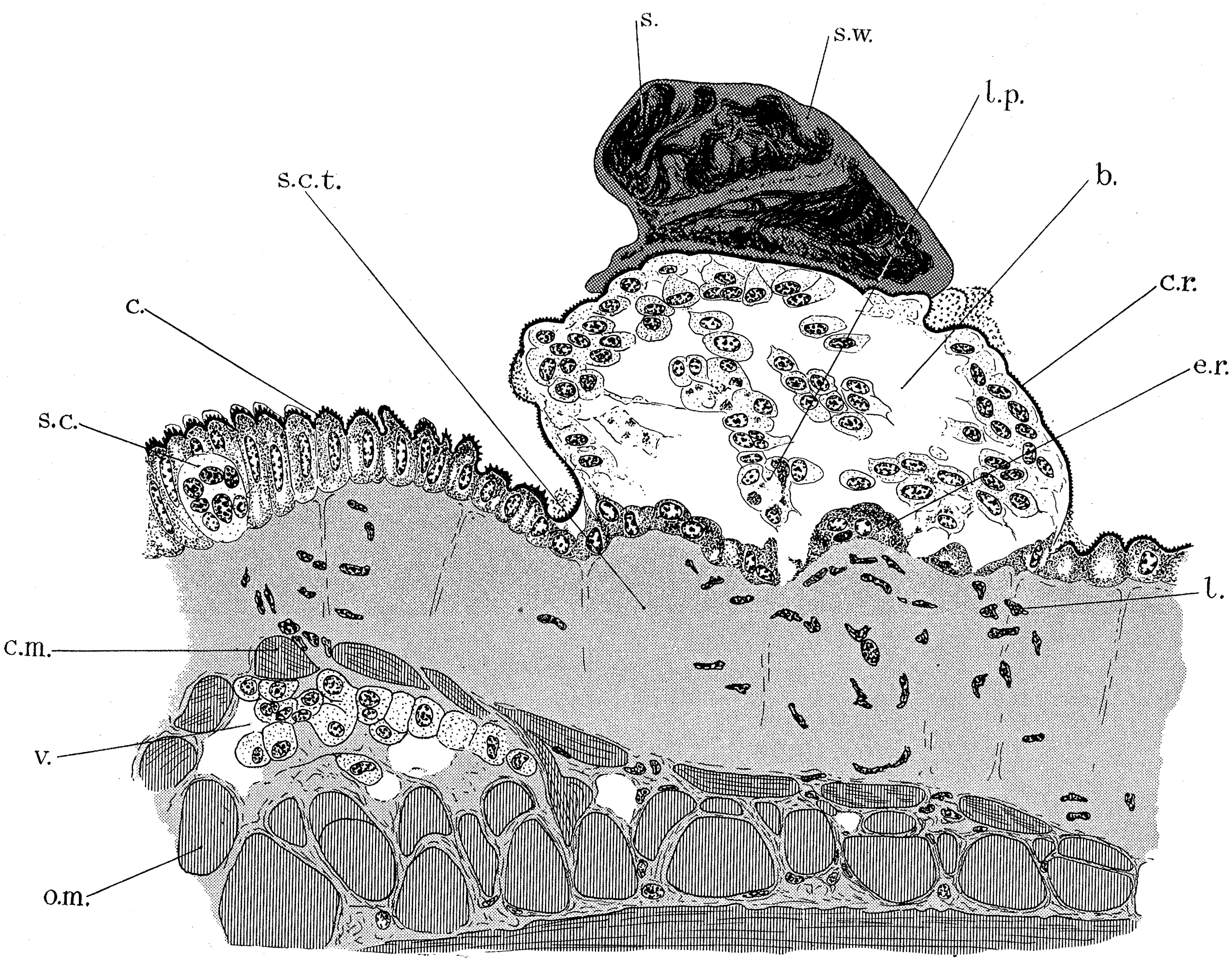

FIG. 2—Vertical section of spermatophore on the body wall seven days after deposition. For description see text. × 324 approx.

leucocytes break through the ectoderm below the spermatophore and accumulate between the ectoderm and the overlying cuticle, which is thereby pushed away from the body. Some ectodermal cells are partially ingested, and within many leucocytes there are patches of pigment granules from the ectoderm cells (fig. 2, *l.p.*). Gaps are thus left in the ectodermal epithelium which communicate with a large blister-like swelling lying below the cuticle and adherent spermatophore. This condition is reached

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seven days after deposition (fig. 2). Externally the elevated spermatophore is still white and glistening.

A short time after the formation of this blister the cuticle and lower wall of the spermatophore become perforated. Whether the cuticle is burst mechanically by the distension of the swelling, or whether the leucocytes directly cause its breakdown is unknown. Spermatozoa then leave the spermatophore and swim actively into the swelling. They pass through the gaps in the ectodermal epithelium and wriggle in between the fibres of the outer layer of connective tissue. From here they spread sideways and inwards reaching the vascular channels of the muscular layers. A small group of spermatozoa in the muscle layer near an empty spermatophore is shown in fig. 4. These spermatozoa are overlying but not penetrating the muscles, and are situated in the loose connective tissue sheaths of the muscle fibres. No sperms were ever found in the tissues near freshly deposited spermatophores full of sperm. Once the integument is perforated the passage of the spermatozoa into the body is rapid, and is completed possibly in an hour or so as it is in some leeches.

After the spermatozoa have left the spermatophore its walls collapse and form a disk-shaped covering to the swelling. Within the latter the leucocytes form a degenerating mass, and those in the connective tissue layer become fewer. Such a stage is shown in fig. 3. The exact age of this very large spermatophore is unknown, but it was more than ten days old (only half of it is shown). Two spermatozoa (*s.*) still remain in the swelling and one in the ectoderm in the section figured. As soon as the spermatozoa pass into the body the spermatophore loses its white appearance and becomes dull and grey.

The degeneration of the leucocytes is followed by the sinking down of the ruptured cuticle and the reorganization of the ectodermal epithelium. The spermatophore wall remains adherent, and thus at all times forms an effective covering of the wound produced by the entry of the sperm. The reorganized ectoderm may be irregular, but it is continuous, and in due course forms a new cuticle below the perforated one. This new cuticle is continuous with that formed elsewhere. Such a stage is shown in fig. 5. The gaps in the old cuticle below the spermatophore wall are clearly seen, while elsewhere the cuticle is double. This animal was about to cast its skin. A few sperm still remain in the subcutaneous connective tissue layer. Externally the flattened empty spermatophore becomes much less conspicuous, and no longer projects appreciably. When ecdysis occurs the ruptured cuticle and adherent spermatophore wall are shed, and the site of penetration of the spermatozoa has a normal or slightly irregular surface.

The passage of the spermatozoa from the vascular spaces of the body to the ovary is probably fairly direct. Spermatozoa are not found in the haemocoel far from the ovary, and their occurrence in connective tissues of the body wall is limited to the vicinity of empty spermatophores. Presumably the spermatozoa are chemically attracted to the ovary. It is believed that they can swim against the blood stream, as they have been

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found in channels in the dorsal part of the pericardial network after leaving a dorsally situated spermatophore. There is reason to believe that blood flows through the pericardial network from below upwards, so that these spermatozoa probably were not carried passively to this position.

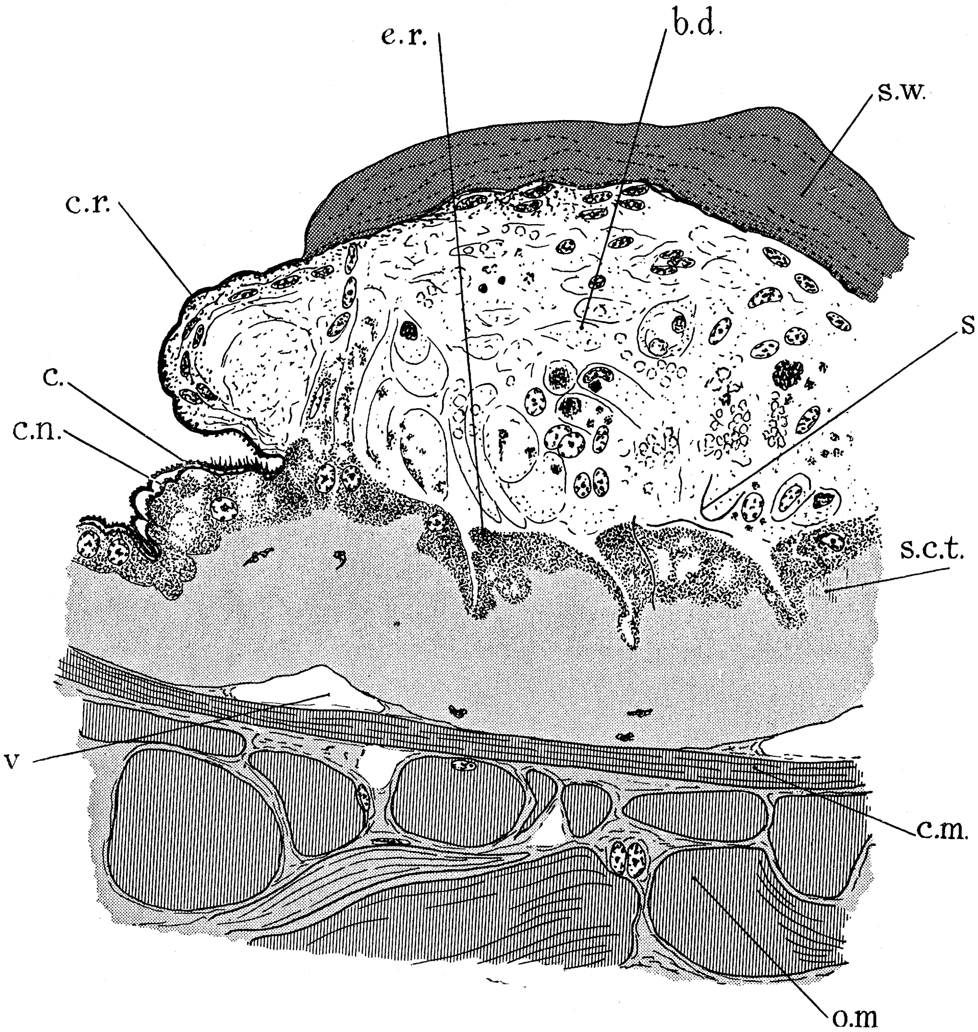

FIG. 3—Vertical section of half a large spermatophore at least ten days after deposition. For description see text. × 392 approx.

The spermatozoa form a dense felt outside the ovary over the region bearing egg follicles (fig. 15, Plate 51, for structure of ovary see pp. 428 and 431). The follicles may barely project through a tangle of sperm heads 50–80μ thick. The outer spermatozoa are free, but the inner ones penetrate the much distended cytoplasm of the outer epithelium (fig. 15, the tails of the spermatozoa are not shown). Groups of these spermatozoa become orientated radially and force their way inwards. The middle layer of connective tissue and muscle is bulged inwards and finally broken through, and clumps of sperm find their way into the germinal epithelium. Here they lie in masses in intracellular spaces, and

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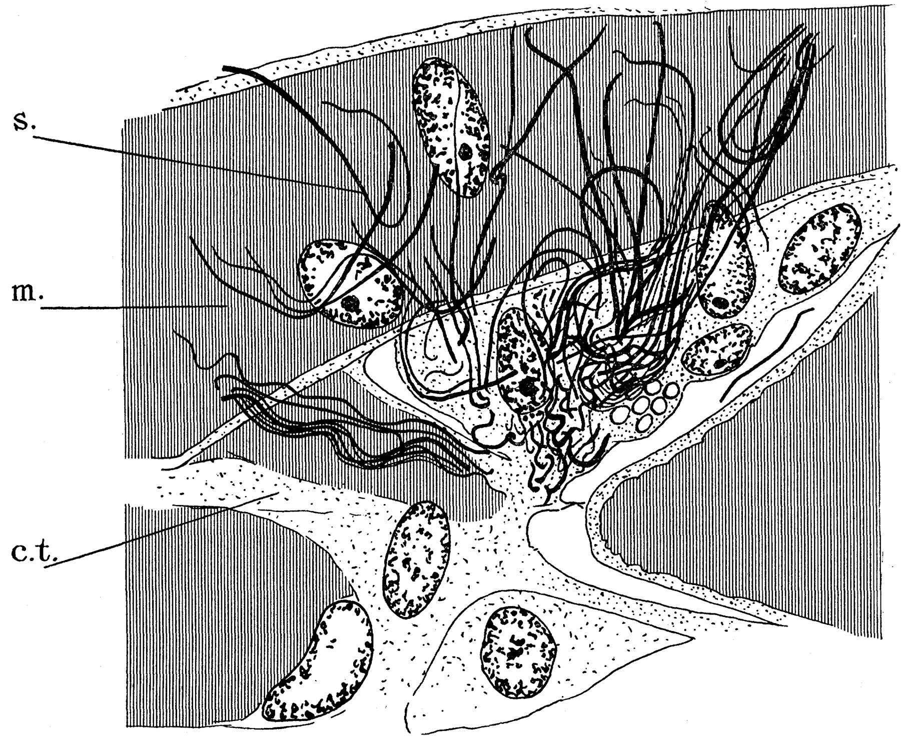

FIG. 4—Section showing a group of spermatozoa (only the heads are drawn) lying between the body wall muscles in the vicinity of an empty spermatophore. × 1204 approx.

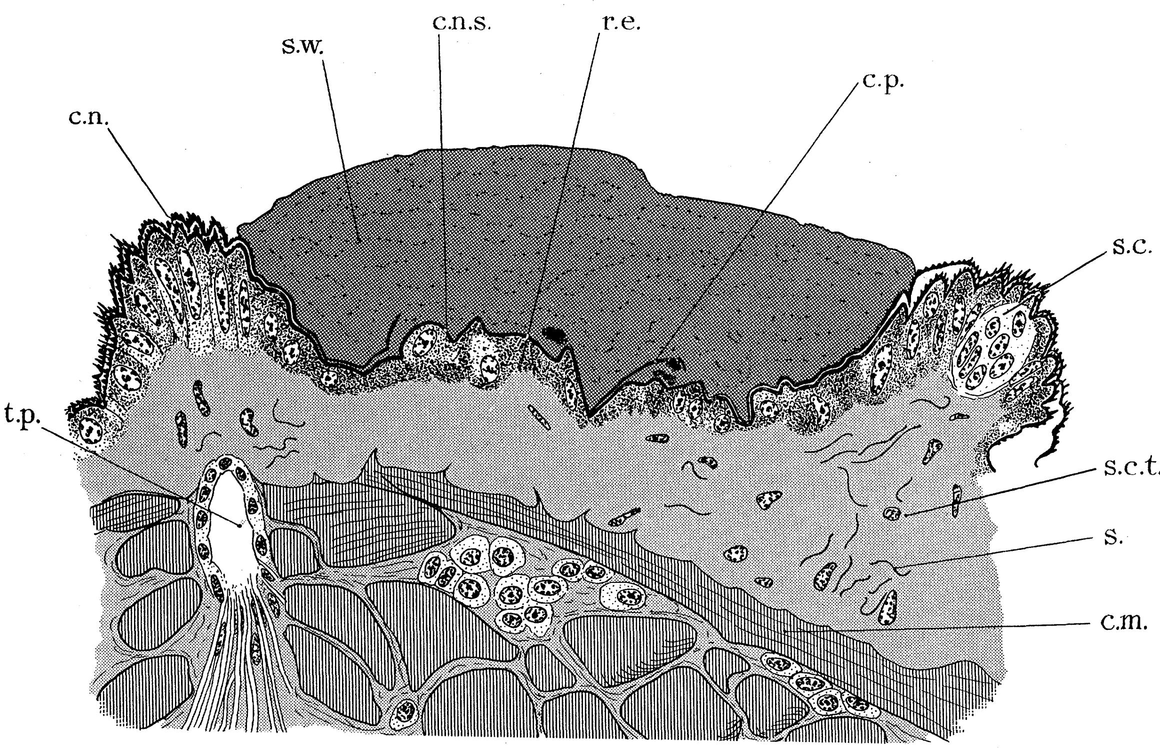

FIG. 5—Vertical section of a spermatophore older than that shown in fig. 3. For description see text. × 350 approx.

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some spread singly through the cytoplasm of these cells. Other clumps of sperm break right through the germinal epithelium and reach the ovarian lumen. An empty channel left by entering spermatozoa is seen in fig. 16, *s.p.p.*, and an entering clump of sperm is shown at *s.p.* The bulk of sperm entering an ovary by this means may be great; figs. 15 and 17 show ovaries on the same scale before and after the immigration of spermatozoa. Only half of the lumen is shown in fig. 17. The disorganization of the germinal epithelium caused by the passage of the spermatozoa may be considerable.

Spermatozoa reach the ovary in this manner during the six months or so in which the male genital organs are functional. The bulk of the spermatozoa may be considerable, and as many as 170 spermatophores may be received by one female during a fortnight.

The Ovary and the Early Development of the Ova

The structure of the paired ovarian tubes in the Onychophora has been described by Kennel (1884), Gaffron (1885), Sheldon (1889), Bouvier (1904), etc. The tissue lining each ovary in *Peripatus* forms a homogeneous germinal epithelium throughout the ovary, while in *Peripatopsis*, *Peripatoides*, and other genera the epithelium is thin and sterile except for a longitudinal thickened ridge of germinal epithelium which projects into the lumen of the ovary. The fixation of the ovaries previously described does not appear to have been satisfactory. No cell boundaries could be distinguished by Sheldon and others, and there is general agreement with Bouvier's statement that "Toutes les cellules épithéliales...de l'épithélium germinatif...semblent identiques et capables de produire un œuf". The earliest recorded ova lie in this germinal epithelium. The later development of the ova in *Peripatus* (where they remain small) takes place entirely within the germinal epithelium, while in the other genera where the ova become larger the later stages are passed in follicles or ovisacs, projecting from the ovary into the haemocoel.

Well-preserved material of *Peripatopsis sedgwicki*, *P. moseleyi*, and *P. balfouri* has been compared with the sections prepared by Sedgwick and Sheldon, and has considerably extended our knowledge of the structure of the ovary and of the development of ova. The use of Mallory's triple stain was invaluable for ascertaining the extent of the connective tissue in the ovary and for distinguishing between various egg membranes. Cell limits are perfectly definite, and the germinal epithelium is not a spongy syncytium. Nuclei are always present in developing ova and do not entirely disappear at certain stages. These two erroneous conclusions of previous workers were due to the methods employed. The characteristics of the development of ova in the Onychophora listed by Sheldon and Bouvier, together with the deductions made, need considerable revision.

The paired ovaries of the various species of *Peripatopsis* are very similar in structure. They are tubular, and continuous with their oviducts; they are always united together posteriorly, and occasionally in other places as well.

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SEDGWICK (1888) described the embryonic ovary of *P. capensis*. He followed the migration of the embryonic germ cells to the dorsal coelomic pouches of segments 16 to 20, and states that "the cells of the latter form capsules surrounding the germinal nuclei". He figures the ovary as a tube with large germ cells distending the thickened inner germinal ridge.

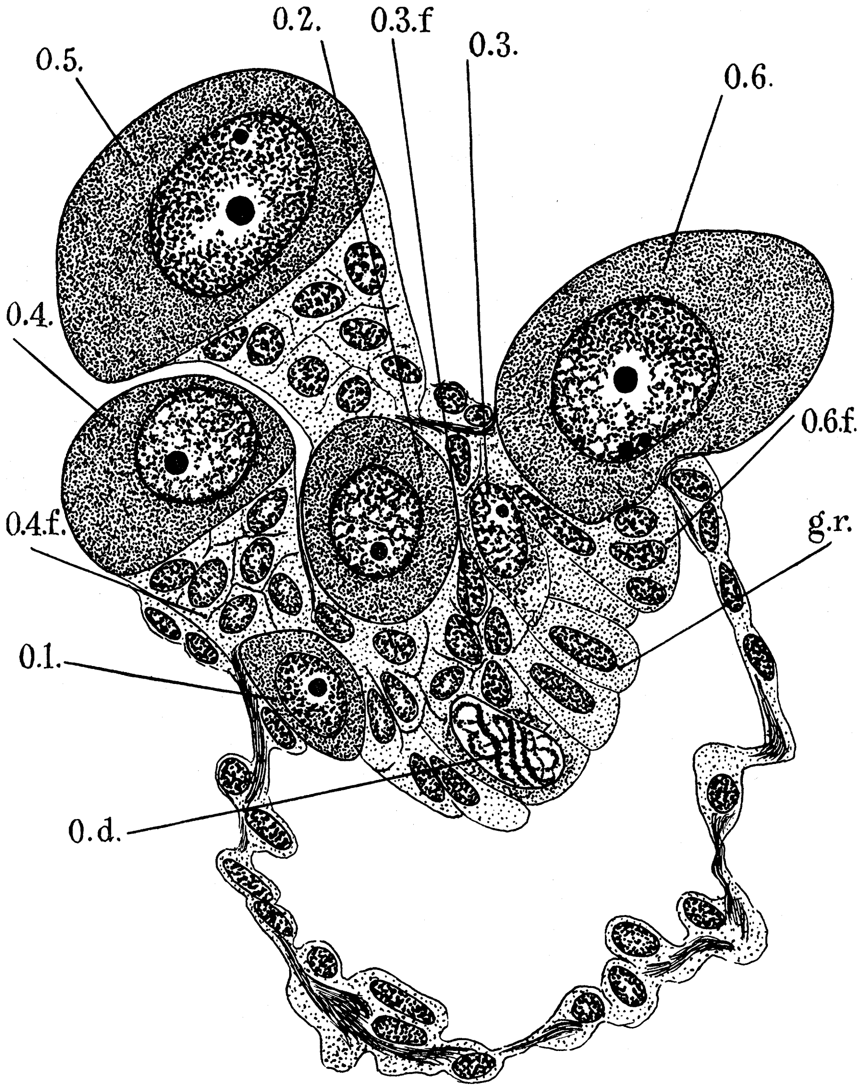

FIG. 6—Transverse section of one ovarian tube of a recently hatched *P. balfouri*. No ova or oogonia lie in the ovary lumen, both are seen only in the germinal ridge. Two ova are already extended on follicles, but the egg membrane is not yet formed. × 980.

The germ cells are at a later stage in the young of a number of species of *Peripatopsis* which have been examined just before and shortly after birth. All these ovaries show the germ cells in the germinal ridge to be quite distinct from the surrounding epithelial cells, just as figured by SEDGWICK. Many of the smaller germ cells, however, are now undergoing fission (fig. 6, *o.d.*) and others have increased in size. Each of the larger ova

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is associated with a group of small follicle cells (fig. 6, *o.*6., *o.*6.*f.*, *o.*3., *o.*3.*f.*, etc.), and the largest ova project from the ovary into the haemocoel, the follicle cells forming a stalk. It is to be noted that all the ova are situated either in the germinal ridge or projecting externally from it in follicles, and that there are no cells of any kind free in the lumen of the ovary. The ovary is also devoid of projections other than the egg follicles. At the age of two days *Peripatopsis* possesses ova resembling in size and all other respects the stage shown in fig. 18, Plate 51, drawn from an ovary of a full-grown animal.

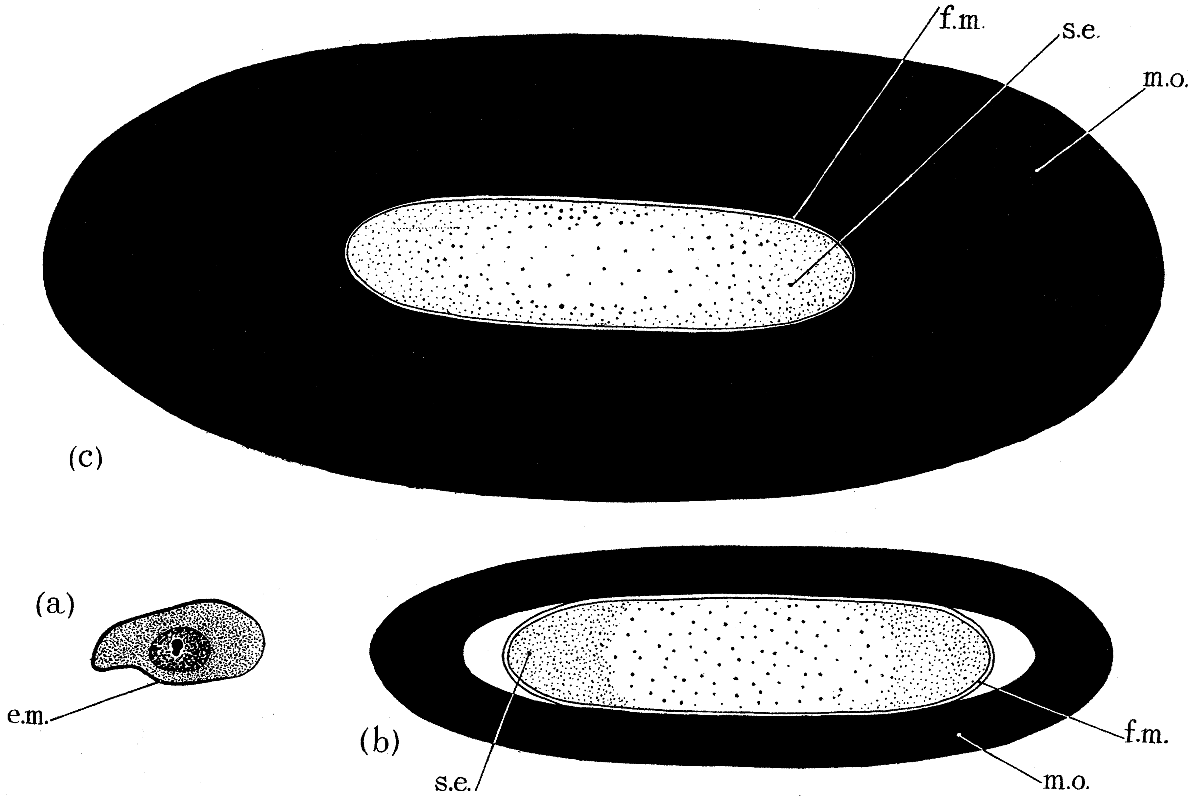

FIG. 7*a*—Ripe ovum from the lumen of the ovary of *P. sedgwicki*. The ovum has just vacated an egg follicle and the egg membrane is very thin at one end prior to the egg emerging from this membrane for fertilization. × 246 approx. (See also ripe ovum in fig. 19, Plate 51).

FIG. 7*b*—Diagram of a living 4–6-celled stage from the upper oviduct of *P. sedgwicki*. The embryo has swelled greatly (cp. fig. 7*a*) and is now enclosed in another membrane (*f.m.*) (possibly a fertilization membrane) and is surrounded by a second thick membrane secreted by the oviduct (*m.o.*). × 246 approx.

FIG. 7*c*—Diagram of living early segmentation stage of *P. sedgwicki* slightly older than the preceding. The segmenting egg is about the same size but shell secreted by the oviduct is much thicker. × 246 approx.

Up to the age of 6–10 months the ovary maintains the same appearance, except that the ova projecting in follicles may be larger in the older animals. After an animal has copulated towards the end of its first year, the course of the early development of ova changes. This new method continues throughout adult life, and will be described in detail below.

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The structure of the ovary of an adult animal differs slightly from that of the young animal which has not copulated. Each adult ovarian tube is formed by (1) an outer irregular epithelium which covers (2) a connective tissue layer bearing muscles running in various directions, and within this lies (3) the inner epithelium. Tracheae are absent from the ovary although the oviduct is abundantly supplied. The sterile parts of the inner epithelium are usually not more than one cell thick, but may be irregular (figs. 15, 17, Plate 51). The thickened ridge of germinal epithelium, which is ventral in the embryo, lies in the adult on the side of the ovary remote from its fellow, and varies greatly in its extent and thickness. Tall columnar cells form pad-like projections which may be many cells thick, and tongues of connective tissue may extend inwards from the middle layer to support these thickenings.

The sterile region of the ovary wall may show short diverticula projecting outwards. These lobes have little or no muscle, they are thin-walled and often filled with sperm (figs. 15, 17, *s.o.d.*). Outside the germinal ridge the ovary wall may bear many and varied projections in animals which have previously given birth to young; many of these are indirectly due to the formation of the projecting follicles which are ultimately vacated by the ripe ova, and will be described below (p. 435). The lumen of the ovary may be large or small, varying with the abundance of contained spermatozoa.

At the age of a little less than one year, when the lumen of the ovary has been filled with sperm, strands of cells from the germinal ridge migrate into and around the sperm mass. These cells become detached from the ovarian wall, take on a round and undifferentiated form, and lie freely in the lumen among the sperm. Later they give rise to ova. The origin of these free oogonia has been observed clearly in only one individual aged about 11 months. Free oogonia together with all stages of development of mature ova from these cells have been followed in many full and partly grown animals of two or more years in age. Whether the oogonia pass into the lumen of the ovary repeatedly throughout life, or only during the first year, is not known. Some germinal epithelial cells become detached at the times when spermatozoa penetrate through the ovary wall, a process causing some disruption of the tissues, but their fate is unknown. In some ovaries free cells are found which resemble blood leucocytes in size and staining reactions, and which may show a lobed nucleus as do many of the leucocytes (see Manton and Heatley 1937, fig. 23). These leucocyte-like cells have never been seen to contain sperm heads.

The youngest oogonia observed in all adult animals examined were lying freely in the lumen of the ovary. None were found in the germinal epithelium, their position in late embryos and during the first few months after birth. Free oogonia occur either singly or in masses, but are always separate from the wall of the ovary. The oogonia may be abundant in parts of an ovary, several dozen touching one another being apparent in one transverse section, and in other parts of the same ovary they may be few and scattered. The oogonia are surrounded by sperm as in fig. 17. The smallest oogonia are almost indistinguishable from free epithelial cells, and have been seen most

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abundantly in ovaries of *P. moseleyi* in February and of *P. sedgwicki* in March and May. This extensive range of months is probably associated with the breeding season of these two species being less restricted than it is in *P. balfouri* and *P. capensis*, the young in the two former species being born from June to September and July to December respectively.

The oogonia are spherical, unless distorted by pressure, and are about 18–20μ in diameter. The cytoplasm is evenly granular, and the nucleus, with characteristic nucleolus, is about 8–10μ across. The nuclei of the oogonia are usually quite distinct from those of the germinal epithelial cells where they are various in size, and the nucleolus, if present, is smaller and less conspicuous (fig. 13, Plate 50, *g.r.n.*). The cytoplasm of the oogonia becomes packed with sperm heads. The length of the head is about 75–100μ (fig. 14), and thus each is folded or curled round the nucleus of the oogonium. It was impossible to count the number of sperm heads within an oogonium, but there must be several. Those shown in figs. 8–10, lie in one plane only, and many others were present on either side, but they have been omitted from the figures for the sake of clarity. Some of the sperm heads appear partly to lie outside the oogonia. Probably these are actual cases of penetration of the oogonium by a spermatozoon, but the abundance of sperms outside the oogonia as well as inside, and the great length of the sperm head which may extend through ten sections, makes a critical statement impossible. The oogonia divide to give descendants like themselves. During cell division the sperm heads lie in the peripheral cytoplasm outside the mitotic figure, and do not appear to hinder this process. Two daughter nuclei are seen in fig. 9. This stage is followed by division of the cytoplasm, and the sperm heads are shared between the daughter cells.

The young ova so formed in the lumen of the ovary increase in size to an average diameter of 32μ in *P. sedgwicki*, but sizes up to $54 \times 40 \times 40\mu$ have been found. The nucleus increases to about 20μ or more, but its appearance is otherwise unaltered. The ova are not further invaded by sperms although they are still surrounded by them, and the contained sperm heads gradually become fewer and finally are entirely absorbed. Stages of this process are shown in figs. 10–12. Some oogonia and early ova show spherical regions in the cytoplasm which stain light or dark blue with Mallory's triple stain (figs. 9, 10). These patches disappear as soon as the sperm heads are all absorbed. Probably they represent some of the material of the sperm head in the process of absorption. In the bed-bug the absorption of sperm heads leaves stainable zones within the absorbing cell (Abraham 1934).

The ova now become attached singly to the thickened germinal ridge. They adhere to the exposed ends of the epithelial cells (fig. 12), and in their vicinity a number of small "follicle" cells (*f.c.*) appear with darkly staining nuclei and cytoplasm less dense than elsewhere. These cells spread round and over each ovum and finally enclose it (fig. 13). The ovum at this stage is about 32–45μ in diameter in *P. sedgwicki*. The follicle so formed usually separates the ovum from the spermatozoa in the lumen of the ovary,

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as in fig. 13, but in some cases a thin but dense investment of spermatozoa lies round the ovum inside its follicle, although these do not penetrate the egg.

The ovum with its follicle cells now sinks farther into the thick germinal epithelium, and comes to lie against the connective tissue layer which separates the inner ovarian epithelium from the outer epithelium. The follicle cells are now situated mostly on the side of the ovum facing the ovarian lumen (fig. 18, Plate 51), the reverse of their original position. The ovum is pushed through the layer of connective tissue and muscle, followed by a plug of follicle cells, and reaches the outer epithelium. This stage represents the earliest one found by previous workers (see Sheldon 1889, Pl. 29, fig. 3), who could not distinguish cell limits in their material and thus could not follow the details of ovary structure and the movements of ova. The size of the ovum increases during this migration to an average of 38μ diameter in *P. sedgwicki*, but smaller and larger specimens have been found measuring $30 \times 30 \times 25\mu$ and $54 \times 34 \times 48\mu$ respectively.

The follicle cells now swell and increase in numbers, and force the ovum away from the general ovarian surface. The beginning of this process is seen in fig. 18 where the ovum is about 38μ in diameter. A thin layer of connective tissue is left covering the ovum as a result of its passage through the ovarian wall, and this connective tissue forms a permanent investment to the projecting follicle, and remains continuous with the main connective tissue layer. Some of the follicle cells shown in fig. 18 still lie in the inner epithelium. These gradually become external and ultimately form a stalk about 120μ long in *P. sedgwicki*. This follicular stalk is only one cell thick (20–30μ) and is flat and wide (70–80μ) (see fig. 17).

When the follicle stalk is fully extended a change takes place in both follicle and ovum. The terminal follicle cells lying against the ovum cease to resemble the rest, and form a regular darkly staining layer, and a space appears between this layer and the opposing wall of the ovum. The latter usually becomes flattened on this side, and acquires a thick membrane staining blue with Mallory and brownish with iron haematoxylin. This membrane has been recorded by Sheldon as the "egg-shell", and others have assumed that fertilization is effected through it, and that it persists into embryonic life. The egg with its membrane then increases in size to a maximum of 70–80μ in diameter in *P. sedgwicki*. There is no evidence to support Bouvier's suggestion that the ovarian egg grows to 600μ in this species. In *P. moseleyi* the follicular egg reaches 150μ in diameter.

The egg remains in this position until 1 or 2 months before the birth of the uterine embryos. The follicle and connective tissue sheath may shrink away from the egg membrane all round, and the ovum then becomes spherical. The nucleus may become greatly distended so that it is not readily distinguishable from the cytoplasm, but it never disappears.

Two specimens of *P. sedgwicki*, "A" killed in January and "B" killed in March, and one specimen of *P. moseleyi* killed in February showed advanced embryos in the lower oviduct, while the upper oviduct contained the earliest embryonic stages recorded for

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these species. In *P. sedgwicki* "A" about a dozen 4–6-celled stages lay in the upper oviduct, and the ovary lumen contained a few unfertilized eggs which had not yet passed into the oviduct. In *P. sedgwicki* "B" the upper oviduct contained early segmentation stages, and the ovary lumen was devoid of mature ova. In both animals all the projecting ovarian follicles were empty, and therefore ovulation must have been completed. In the *P. moseleyi* only two unsegmented eggs lay in the upper oviduct, and the ovarian follicles showed a few ova; thus in this animal ovulation had only just begun.

The further history of the ova after their growth in the follicles appears to be as follows: over the whole ovary the cells of the follicle stalks alter their arrangement and form hollow tubes instead of flat plates. Each ovum covered by its thick membrane passes through the follicle stalk into the ovarian lumen, going through the gap in the connective tissue through which it previously came. The germinal epithelium may be disorganized by the passage of the large ovum; and the follicle remains as an empty diverticulum of the ovary (fig. 19). The ova in the ovarian lumen in *P. sedgwicki*, specimen A, measure 65–80μ in diameter and most are slightly elongated. They are surrounded by spermatozoa and by cells, partially or completely detached from the germinal epithelium. The egg membrane is entire and lacks a micropyle, and the nucleus of each ovum is so far unaltered (fig. 19).

The ovum then swells slightly, and the egg membrane becomes thin and stretched at one end (fig. 7*a*). It is believed that the ovum then emerges from its membrane. Empty membranes have been found in the ovarian lumen corresponding roughly with the number of young segmentation stages in the upper oviduct, but neither could be counted exactly. It is probable that the naked egg is then fertilized by a spermatozoon, and that it rapidly passes into the oviduct.

The next stage seen was also found in the females mentioned above. The 4-celled egg in *P. sedgwicki* had swollen to $260 \times 80 \times 80\mu$ (fig. 7*b*), and the unsegmented egg in *P. moseleyi* measured 520μ. This sudden swelling was noted by Sheldon for *P. capensis*, but it was ignored by Bouvier whose conclusions in consequence are erroneous.

The fertilized and segmenting eggs in the upper oviduct are covered by two membranes. A thin inner membrane stains red with Mallory, in contrast to the ovarian egg membrane which stains blue. An outer membrane is progressively secreted by the oviduct wall and rapidly attains the enormous thickness of 140–240μ in *P. sedgwicki* and stains blue with Mallory (fig. 7 *b* and *c*). This outer membrane stretches as the segmenting egg swells to a length of 2·5–3 mm., and remains intact until shortly before the birth of the young. The substance of the egg within the inner membrane is at first very diffuse owing to the rapid swelling, and masses of protoplasm containing chromosome or nuclear spheres lie scattered through the fluid contents. Sedgwick had the good fortune to kill an animal at a stage in which the upper oviduct ova were showing maturation and fertilization phenomena (Sheldon 1889). His stages are intermediate between the ovarian and uterine ova from specimen A of *P. sedgwicki* described above. Sheldon's

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preparations have been re-examined and they show that the maturing egg with its male and female pronucleus is surrounded by a membrane stained with haematoxylin which she did not figure. It is probable that this single membrane covering the egg after fertilization is the inner membrane of the enlarged segmenting egg shown here in fig. 7*c* which also stains with haematoxylin (a blue-black colour). It is certainly not the membrane covering the ovarian egg, as this is left in the ovary and has different staining properties, appearing blue with Mallory while the inner membrane of the uterine egg stains red. The latter may be a fertilization membrane. It disappears later when the segmenting egg has increased to a length of 2·5 mm.

The emptying of all the ovarian follicles at ovulation leaves the ovary covered with empty and shrunken diverticula. These may become more or less solid projections which remain on the ovary (fig. 15, Plate 51), or they may be invaded by few or many spermatozoa from the lumen of the ovary. The proximal parts may be distended by sperm and then the distal regions become solid or shrink, or the distal parts may become distended, the wall remaining thin; the whole then externally resembles an egg-containing follicle, and may even exceed the latter in size. Most of the spermatozoa remain in the lumen of these lobes, but a few penetrate the follicle cells.

It is not known whether these effete follicles are ever completely absorbed. Some of them may be, but an old animal shows many more sterile than fertile follicles attached to its ovaries. The passage of the ova from the follicles to the ovarian lumen is followed by fresh ova becoming attached to the germinal epithelium, and the subsequent formation of new follicles in the manner described.

The number of follicular ova on one ovary varies with the condition of the animal, a feeble starved specimen sometimes showing fewer ova than normal. In all well-fed individuals the number of follicular ova exceeds the number of segmenting eggs in the upper oviducts. The latter correspond closely to the number of embryos in the lower oviducts. If all the follicular ova pass into the ovary at ovulation, as is suggested by the specimens examined, some of these ova must fail to give rise to segmenting eggs. The fate of such surplus ova is unknown. Absorption of germ cells occurs in other animals (e.g. *Hemimysis*, see Manton 1928, but here it takes place at an earlier stage).

In all adult animals examined the oogonia and early ova contain sperm heads in their cytoplasm at stages younger than that of fig. 12, Plate 50. Whether in adult animals the ova can develop and give rise to normal eggs in the absence of spermatozoa, as they do in newborn and very young animals, or whether their growth is entirely dependent upon the sperm they normally ingest is not definitely known. Examination of one animal indicates that the latter may be the case. A 3 or 4 year-old *P. sedgwicki*,* which had previously produced normal young, and which contained late embryos in the oviduct, refused to feed and was dying of starvation. On killing, the ovary was found to be devoid of sperm, although copulation had been frequent. Free oogonia and young ova, which normally contain sperm heads, were absent, and only a few ova, not more

* *Peripatopsis* normally lives for at least six years and reproduces up to at least the sixth year.

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than seven, lay in the germinal ridge.* A reduced number of fertile ova had just passed into the upper oviduct. In this case it appears that (1) the animal had absorbed ovarian spermatozoa when food was not eaten, and (2) that the development of young ova ready to penetrate the germinal ridge had not taken place normally in the absence of abundant ovarian spermatozoa. Thus a certain number of spermatozoa may be necessary for absorption by the ova and oogonia of adult animals.

The sequence of events described above for the development of ova is quite definite. A complete series of stages has been examined for *P. sedgwicki*; the free ova and all the follicular stages have been seen in *P. moseleyi*; naked ova becoming attached to the germinal ridge (as in fig. 11), and all subsequent follicular stages have been seen in *P. capensis*; and follicular stages alone have been examined in *P. balfouri*.

The duration of the various stages of ovum development is less certain. The follicular growth stages of *P. capensis* described by SHELDON (1889) as occurring progressively during a period of about 9 months from July to the following April, correspond with those of *P. sedgwicki* starting at a stage a little younger than that shown in fig. 18. It is probable, for the reasons given below, that the duration of ovum development is not rigidly fixed or always so long, and that in *P. sedgwicki*, *P. moseleyi*, and *P. balfouri*, and probably in other species as well, the development of ova ready for fertilization may occur quite rapidly instead of taking more than 9 months.

1—Some individuals of *P. sedgwicki* and *P. moseleyi* killed from January till July show all stages between early oogonia and stalked follicles bearing full-size ova on the same ovary. Thus these stages here do not appear to be reached progressively at certain months in the year in any one animal. Only in ovaries from animals which had recently given birth to young were a limited number of stages found. Here all the ova in the germinal ridge and on follicles were small and young, and a relatively small number of follicular ova had so far been formed.

2—The ovary of a *P. balfouri*, killed in June and carrying segmentation stages in the oviduct, showed all stages of follicular ova, but no free ova or oogonia in the lumen. If all the ova pass from the follicles at ovulation in *P. balfouri* as they do in the specimens of *P. sedgwicki* here examined, then the fully formed follicular ova in June must have developed since the last ovulation, a period of about 2–3 months, and unless they are absorbed must remain in this state until the next ovulation, which will occur in about 9–10 months' time.

3—Projecting follicular ova are well developed in animals a few days after birth. Such ova are absent in advanced embryos, and thus must develop in a few weeks or days.

It is thus probable that the development of ova from oogonia to fully formed follicular stages may take place both rapidly and at various times in the year, and that the ova surrounded by a membrane on the projecting follicles may remain dormant in this position for several months before passing back into the ovary for fertilization.

* The ova in or attached to the germinal ridge are normally too numerous to count.

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The Absorption of Spermatozoa in the Ovary

It has been noted above (p. 428) that the bulk of spermatozoa reaching the ovary during the year may be great. It is unlikely that the large mass of spermatozoa which may be found in the ovaries of *P. sedgwicki* or *P. moseleyi* is ever completely absorbed at one period during the year. No ovary was devoid of spermatozoa in animals aged 6 months and upwards. Sedgwick (1885) noted that spermatozoa occur "in smaller numbers directly after the eggs have passed into the oviduct than at any other time". Sedgwick's preparations have been re-examined, and they show a much smaller accumulation of spermatozoa than is found at times in the species here described (see fig. 17 and compare with Sheldon 1889, Pl. 30, fig. 22).

It is evident that a large amount of spermatozoa disappear from the ovarian lumen, but the manner of utilization of so much sperm is not fully understood. An insignificant number fertilize the 20–60 mature eggs, and a greater number penetrate the developing oogonia and are absorbed by them, but the extent of such absorption cannot be estimated. It is probable that the majority of spermatozoa are absorbed by the ovary in some other manner. The cells of the inner ovarian epithelium frequently contain single sperms within their cytoplasm or clumps of sperm lying within large vacuoles, but there is no indication as to how far these sperm are in process of penetration to the lumen or being absorbed by the epithelium. It has already been noted that during starvation absorption may be abnormally great, so that although copulation is frequent the ovary may be devoid of sperm.

Discussion

Fertilization in many groups of invertebrates is obtained by the passage of spermatozoa through the body wall of another individual. Spermatozoa may be injected anywhere into the body by a muscular penis, as in *Dinophilus*, Rotifers and many Turbellaria. In the bed-bug *Cimex* the spermatozoa pass through the body wall by their own activity, making their way through the cells of the organ of Berlese to reach the haemocoel. In other invertebrates spermatophores may be employed as a means to internal fertilization. In leeches such as *Clepsine* and in *Peripatopsis* closed spermatophores are placed anywhere on the body, and their contained spermatozoa pass through the body wall. In many Ichthyobdellid leeches a specialized "tissu vecteur" is provided in the clitellar region for the passage inwards of the sperm (Brumpt 1900).

Peripatopsis thus resembles *Cimex* and *Clepsine* in that the spermatozoa from the spermatophores pass into the body by their own activity, but the details of this process in *Peripatopsis* are unlike those in any other animal described. In *Cimex* a cytoplasmic surface is provided by the organ of Berlese, while in *Clepsine* and *Peripatopsis* the spermatozoa have to traverse the cuticle and body wall. In *Clepsine* (Whitman 1891) and *Placobdella* (Meyers 1935) this is a rapid process, and starts a few minutes after deposi-

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tion of the spermatophore. Spermatozoa stream through the skin for about one hour, and the waterproof spermatophore wall contracts and appears to force the sperm into the body. A small initial perforation of the cuticle may or may not be made by the hard spermatophore on deposition (MEYERS 1935). The rapid perforation of the body wall is caused by a secretion of the epididymis which causes histolysis of the tissues in the neighbourhood of the spermatophore attachment. In *Peripatopsis*, although the integument is very thin, the passage inwards of the sperm is delayed for 7–10 days until leucocytes have caused sufficient breakdown of the ectoderm and integument to allow the sperm to swim freely into the haemocoel. No further breakdown of the tissues takes place and no cytolysing agent is secreted by the male. Thus the penetration in *Peripatopsis* is very different from that in the leech, and in neither *Peripatopsis* nor *Cimex* do the sperm themselves penetrate through tough membranes.

In *Cimex*, *Clepsine* and *Peripatopsis* the sperms reach the ovary by their own activity, and after traversing the body spaces they penetrate through the ovary wall. In *Peripatopsis* they pass through this wall in clumps rather than singly, just as described by CRAGG (1920) for the organ of Berlese of *Cimex*. In *Cimex* the spermatozoa from the haemocoel penetrate into the "resorptions Organe" (previously called "spermathecae", see ABRAHAM 1934), and some of them pass on up the ovariole wall intracellularly and reach the developing ova.

Fertilization of the ova in *Peripatopsis* undoubtedly takes place after each egg has vacated both follicle and egg membrane and is about to enter the oviduct. It is an event completely dissociated from the early absorption of sperm by the cytoplasm of the oogonia and ova. In these early stages the nucleus remains unchanged throughout the period of sperm absorption which is completed weeks or months before fertilization. In *Cimex* ABRAHAM (1934) suggests that polyspermy occurs in the ovariole, and that of the many sperms entering the egg only one effects fertilization, the rest being absorbed. Thus here the egg receives spermatozoa but once. Details of these events in *Cimex* have not been observed beyond the disappearance of spermatozoa near the developing ova.

It was first suggested by CRAGG (1923) that spermatozoa provide nourishment for the developing ova of *Cimex*. When the supply of spermatozoa is becoming exhausted in a well-fed bug the animal ceases to lay normal eggs and produces small abnormal and sterile ones instead. Unless spermatozoa are used to nourish the ova it is difficult to account for the formation of ill-developed eggs in their absence. The only direct observation supporting this suggestion is the continual migration of spermatozoa up the ovariole wall and their disappearance in the vicinity of the developing ova. In the adult specimens of *Peripatopsis* here described, the early development of the ova is associated with the absorption of sperm by the cytoplasm of the ova, and a lack of spermatozoa in the ovary is associated with an absence of young ova. It is thus possible that in *Peripatopsis* as well as in *Cimex* the normal development of ova is dependent upon sperm absorption. In newborn *Peripatopsis* young ova develop directly in the germinal ridge without sperm absorption. This may be regarded as the normal or

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primitive method of egg development which probably preceded the present adult condition where sperms are absorbed.

Copulation in *Cimex* and *Peripatopsis* is frequent, and a great volume of spermatozoa is absorbed and disposed of by the female genital organs of both these animals. It has been shown by Abraham (1934) that most of the spermatozoa in the female *Cimex* are normally absorbed by the "resorptions Organe". Here the sperms become motionless within two days and subsequently disappear. Absorption also takes place in the haemocoel by means of leucocytes. In *Peripatopsis* no special organs are developed for sperm absorption which must take place in the ovary, and in the haemocoel no leucocytes have been seen to contain sperms. The physiological significance of this utilization of spermatozoa is unknown. Spermatozoa are not retained intact by starving females of *Peripatopsis* or *Cimex* (Cragg 1923) which are copulating freely. Here the sperm may be used for supplying nourishment or other substances to the female. In mammals and some other animals a small number of spermatozoa are absorbed by cells of the female genital tract, but this absorption, unlike that of *Peripatopsis* and *Cimex*, has little, if any, physiological significance.

Summary

1. The passage of spermatozoa to the ovarian lumen from the spermatophores attached to the body of the female is described.

2. Following the deposition of a spermatophore, leucocytes invade the subcutaneous region and break through the ectoderm. The cuticle of the body and the lower wall of the spermatophore are ruptured.

3. Spermatozoa swim from the spermatophore through the perforated cuticle and ectoderm and reach the vascular spaces. They pass through the haemocoel by their own activity and reach the ovary.

4. Clumps of spermatozoa force their way through the ovarian wall to reach the ovarian lumen.

5. The spermatophore wall remains attached to the cuticle, so closing the wound caused by the entry of the spermatozoa. The ectoderm regenerates and forms a new cuticle which is exposed at the next ecdysis.

6. The earliest oogonia in adult animals lie freely in the ovarian lumen among the spermatozoa. Their cytoplasm is invaded by sperm heads. They divide to form ova which absorb the sperm heads and grow.

7. Each ovum sinks into the germinal ridge epithelium. The formation of the egg follicle and ovarian egg membrane are described.

8. The ripe ovum passes through the follicle stalk back to the ovarian lumen. Here it emerges from the egg membrane. Fertilization takes place and the egg passes down the oviduct. The fertilized egg swells rapidly and a membrane is formed round it, which is probably the fertilization membrane. The oviduct secretes a second very thick membrane outside the first; only this second membrane persists during embryonic life.

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9. The spermatozoa in the ovary (1) fertilize the eggs, (2) provide the early ova with nutriment necessary for their growth, and (3) may supply the animal with nourishment or other special substances.

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Key to Lettering

b. blister below spermatophores filled with leucocytes.
b.d. blister below spermatophore filled with degenerating leucocytes.
c. cuticle of body surface.
c.m. circular muscle.
c.n. new cuticle of body forming beneath the old one.
c.n.s. new cuticle formed by regenerated ectoderm below the spermatophore.
c.p. cuticle below spermatophore showing perforations.
c.r. cuticle raised up from ectoderm below spermatophore.
c.s. connective tissue layer pushed inwards by invading spermatozoa.
c.t. connective tissue.
c.t.m. connective tissue-muscle layer of ovary wall.
d. diverticulum of ovary wall.
d.g.r. germinal ridge cells displaced by passage of ripe ovum.
e. ectoderm.
e.f. empty follicle vacated by ripe ovum.
e.m. membrane formed round ovum in follicle.
e.r. ruptured ectoderm.
f. stalk of egg follicle.
f.c. follicle cells which will pass out behind the ovum to form the follicle stalk.
f.m. membrane formed round egg after fertilization.
f.o. free young ova or oogonia in the ovary lumen.
g.r. germinal ridge.
g.r.n. nucleus of germinal ridge epithelial cell.
i.p. inner epithelium of ovary wall.
l. leucocyte.
l.o. lumen of ovary.
l.p. leucocyte containing pigment granules from ingested ectodermal cells.
m. muscle.
m.o. egg membrane secreted by oviduct.
o. ovum.
o.d. oogonium preparing for division.
o.f. ovum in projecting follicle and enclosed in egg membrane.
o.m. oblique muscle.
o.p. outer epithelium of ovary wall.
*o.*1., *o.*2., *o.*3. young ova in germinal ridge.
*o.*4., *o.*5. older ova on projecting follicles, egg membrane not yet formed.
*o.*3.*f.* follicular stalk cells of *o.*3.
*o.*4.*f.* follicular stalk of *o.*4.
*o.*6. ovum starting to project from ovary.
*o.*6.*f.* cells which will form follicular stalk of *o.*6.
p. passage through ovary wall left by ripe ovum passing from follicle to ovary lumen.
r.e. regenerated ectoderm below spermatophore.
r.o. ripe ovum free in ovary lumen but still enclosed in ovarian egg membrane.
s. sperm heads.
s.c. sense capsule.
s.c.t. subcutaneous connective tissue layer.
s.e. segmenting egg.
s.g.r. sperms in cells of germinal ridge.
s.l. sterile projecting lobe from ovary.
s.o. free sperms in ovary lumen.
s.o.d. free sperms in diverticulum of ovary lumen.
s.o.p. sperms invading outer epithelium of ovary.
s.p. plug of sperms penetrating through ovary wall which is not yet fully perforated.
s.p.p. open passage left by a plug of sperms which has passed into ovary lumen.
s.w. spermatophore wall.
t.p. tracheal pit with attached tracheae.
v. vascular space in body wall.

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Description of Plates

Plate 50

Sections showing oogonia and young ova of *Peripatopsis sedgwicki*. × 1517 approx.

Fig. 8—Two young ova or oogonia containing sperm heads in the cytoplasm from the lumen of the ovary in May. These cells are surrounded by free sperms and other ova like themselves.

Fig. 9—Oogonium from the lumen of the same ovary in process of division. Nuclear fission is complete and will be followed by that of the cytoplasm. Sperm heads lie in the peripheral cytoplasm, and do not hinder the division. The darkly staining spheres (blue with Mallory) probably represent material from the sperm heads in process of absorption.

Fig. 10—Older ovum from the lumen of the same ovary. Three other ova lie in contact with it, and it is also surrounded by free sperms. The ovum is larger, but contains fewer sperm heads, and the darkly staining zones in the cytoplasm probably represent absorption of sperms. From this stage onwards no further sperms pass into the cytoplasm of the growing ovum, although sperms are in free contact with it.

Fig. 11—Older ovum from lumen of ovary in March. Nearly all the sperm heads have been absorbed.

Fig. 12—Ovum of about the same size as the last which has absorbed all sperms and has become attached to the inner epithelium of the germinal ridge. Many sperms lie in the ovary lumen.

Fig. 13—Ovum at a slightly later stage from an ovary in March. The ovum has sunk into the germinal ridge and become overgrown by follicle cells with small nuclei. The edge of an intra-cellular space in the germinal ridge which is filled by sperm is cut in this section. (s.g.r.)

Fig. 14—Sperm head of average size drawn to the same scale as the ova here figured.

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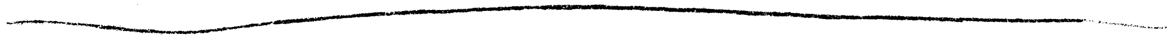

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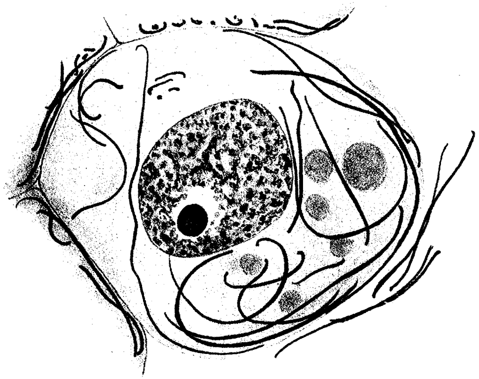

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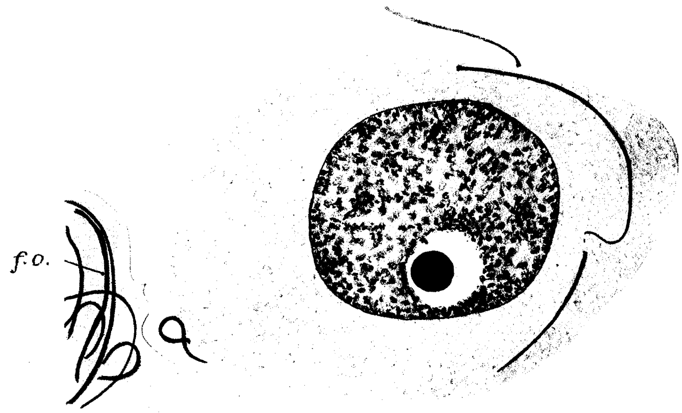

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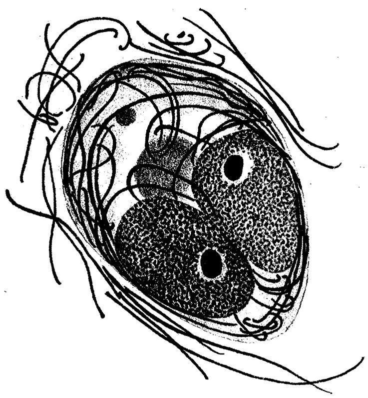

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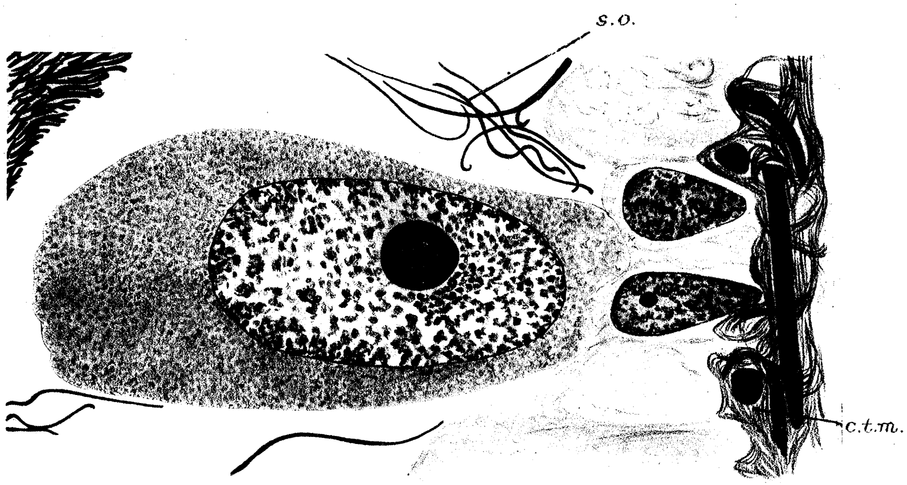

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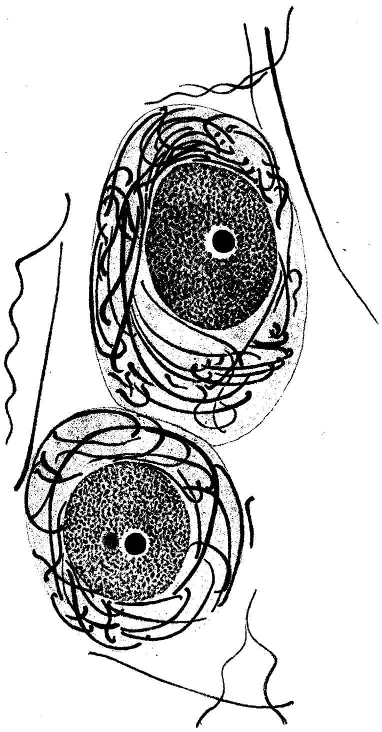

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Plate 51

Sections of ovaries of *P. sedgwicki* showing the penetration of sperms into the ovarian lumen from the haemocoel, and the later development of the ova after they have sunk into the germinal ridge.

Fig. 15—Transverse section of ovary in July. A few very young ova and sperms lie in the lumen. The ovary is moderately contracted owing to the scarcity of solid contents; the germinal ridge projects into the lumen on one side, and externally project many sterile lobes (*s.l.*) and fertile follicles (*o.f.*). The cytoplasm of the outer epithelium of the ovary wall is swollen on the follicle side of the ovary, and is distended with a mass of sperm heads which will later pass into the ovarian lumen. × 290 approx.

Fig. 16—Transverse section of part of the ovary wall in March showing the penetration of sperms through the wall to reach the lumen. A clump of sperm heads (*s.p.*) have pushed in from the distended outer epithelium, and have partly penetrated through the connective tissue-muscle layer of the ovary, which is pushed inwards towards the germinal ridge. Another clump has already passed right through the wall to the lumen (*l.o.*) and has left an open passage (*s.p.p.*) through the connective tissue layer. Many intracellular sperms are seen in the germinal ridge, but the main mass of sperms lies in the ovary lumen outside the region figured. × 490 approx.

Fig. 17—Transverse section of half of an ovarian tube in May. Sperm penetration is now complete. No sperms lie in the outer epithelium or project from the haemocoelic side, and the lumen is distended with a great mass of sperms among which lie a few oogonia or ova. Some sperms lie in vacuoles of the germinal ridge. Two large ova project from the ovary on follicles. The stalks of the follicles are fully formed, flat and one cell thick, and differentiated distally into a darkly staining layer next to the ova. A membrane which stains blue with Mallory has been formed round the ova. Small diverticula of the ovary wall contain sperms from the lumen. × 290 approx.

Fig. 18—Younger follicular ovum from the same ovary. The ovum is of about the same size as in the stage represented in fig. 13. The follicular cells have expanded and pushed the ovum through the connective tissue-muscular layer. The ovum and follicle cells are covered with a thin layer of connective tissue which remains continuous with that of the ovary wall and covers the projecting follicle. No membrane yet covers the ovum. × 290 approx.

Fig. 19—Section of part of the ovary wall in March. All the ova in this ovary have just left the follicles. One ovum is here seen in the ovarian lumen just after it has vacated the follicle (*e.f.*) which is hollow and communicates (*p.*) with the lumen. The germinal epithelium is a little disorganized by the passage of the ovum. The ovum is still covered by the ovarian egg membrane which will be shed, see text-fig. 7*a*. × 290 approx.

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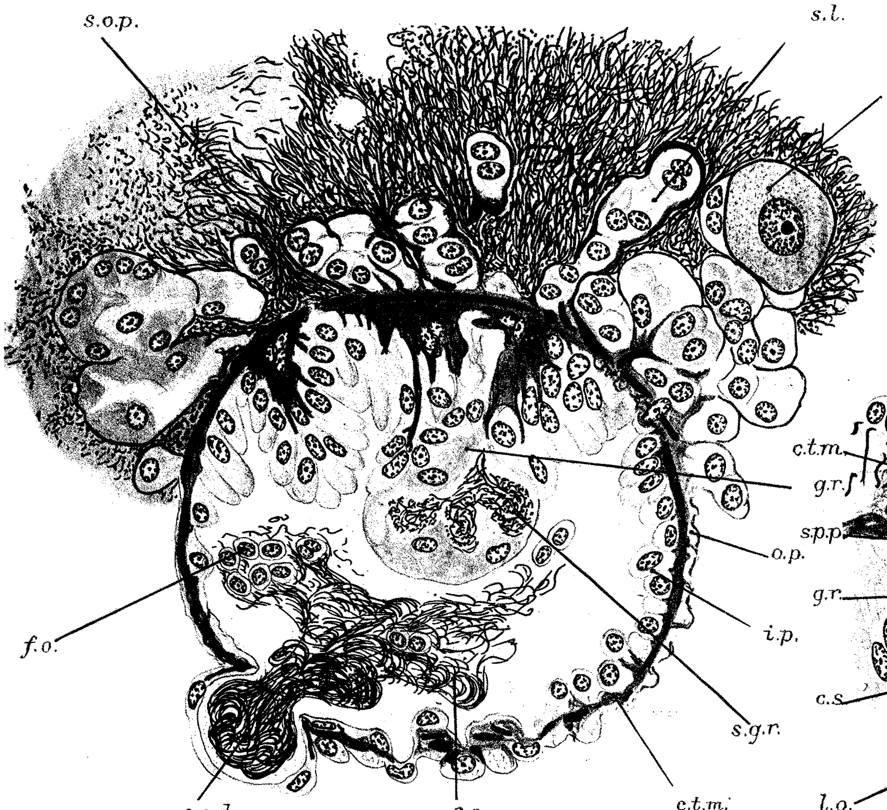

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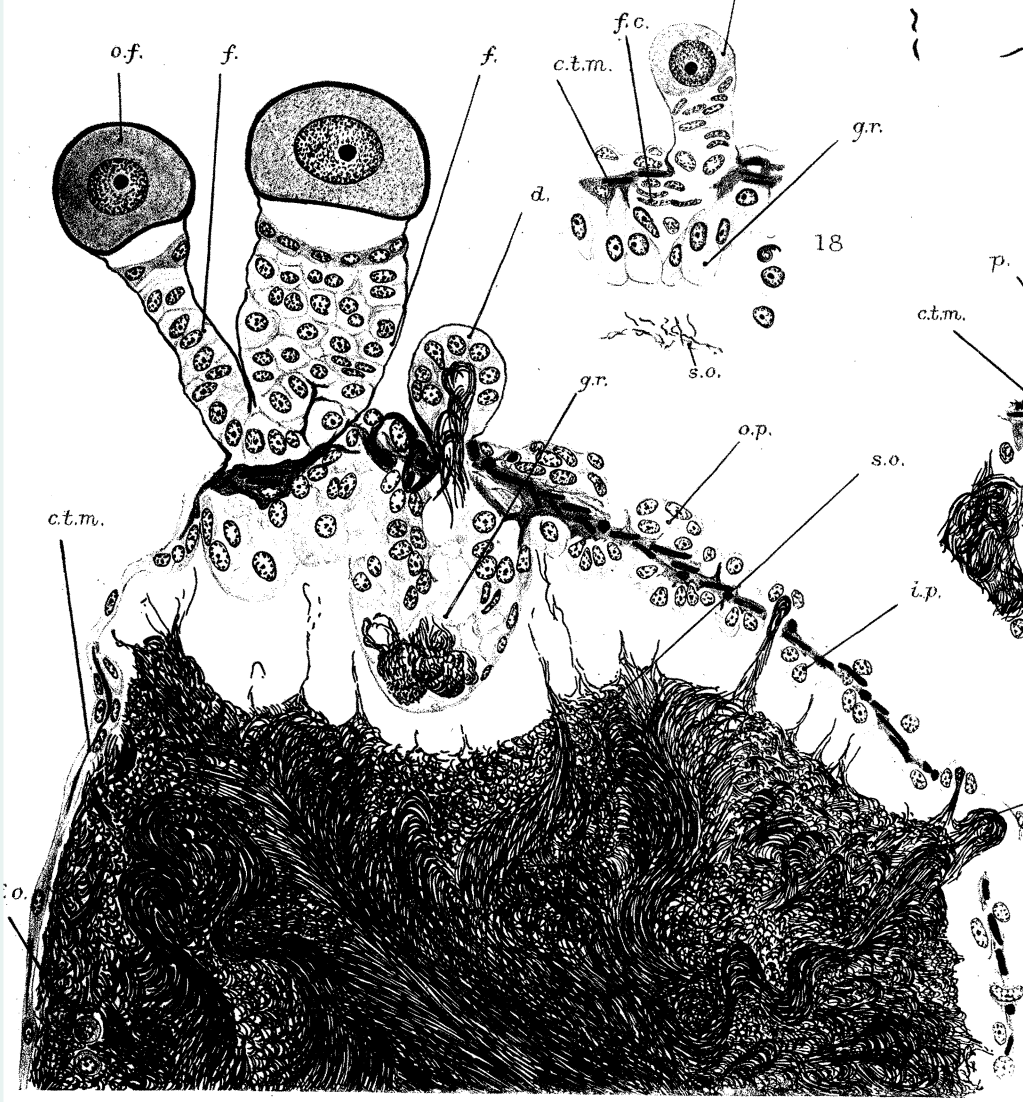

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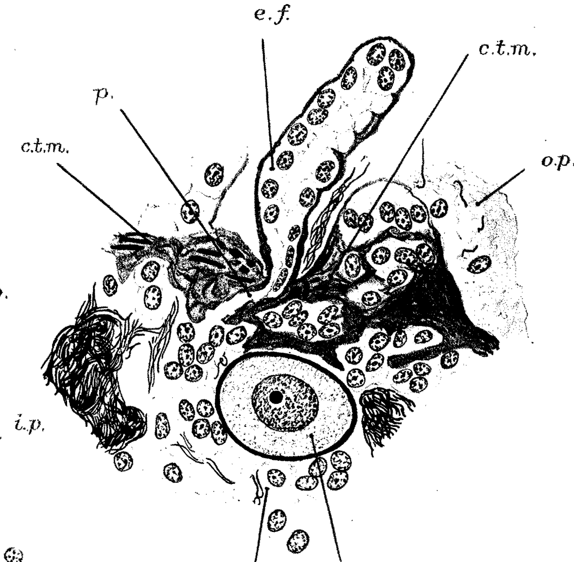

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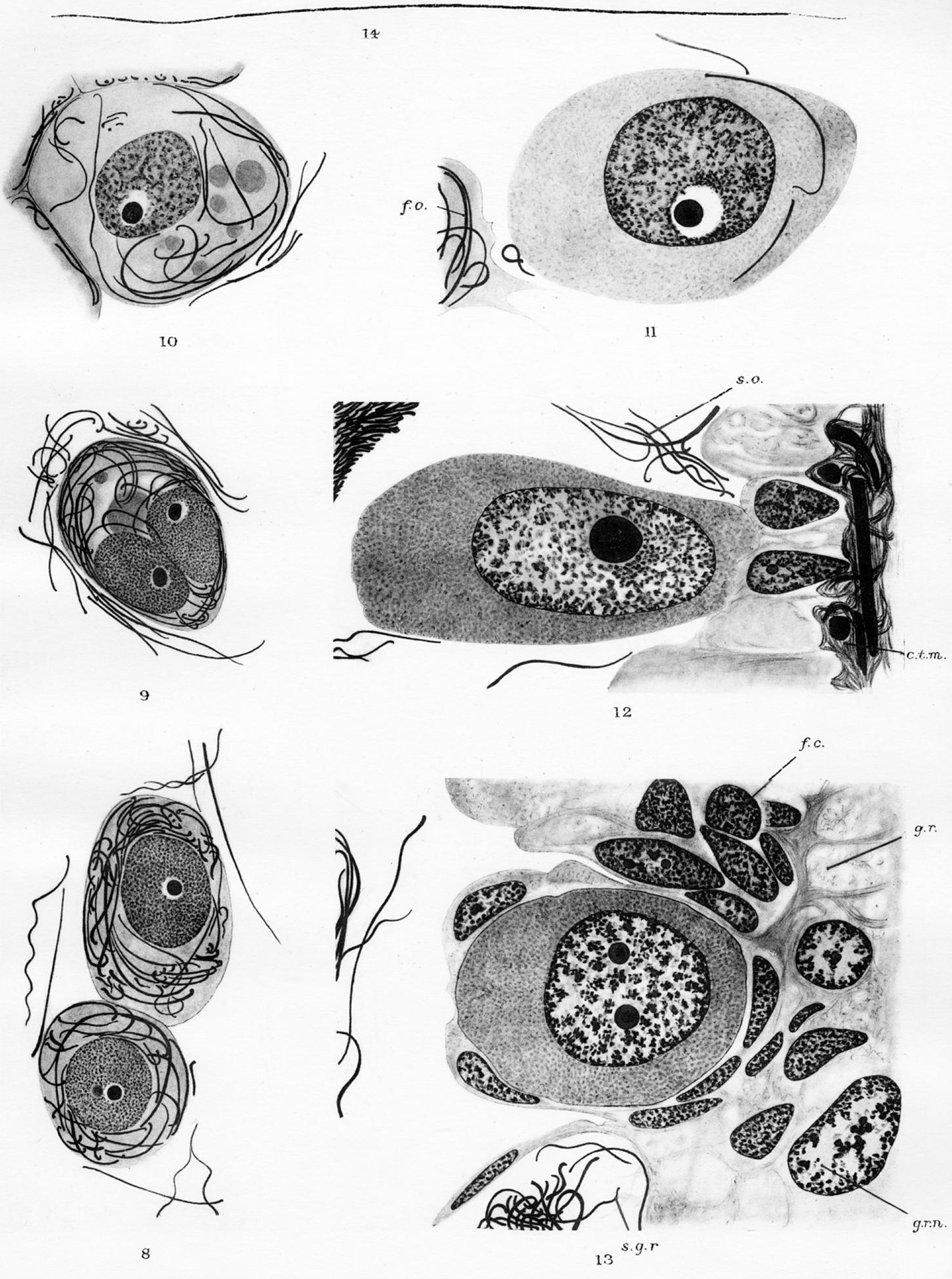

PLATE 50

Sections showing oogonia and young ova of *Peripatopsis sedgwicki*. × 1517 approx.

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FIG. 8—Two young ova or oogonia containing sperm heads in the cytoplasm from the lumen of the ovary in May. These cells are surrounded by free sperms and other ova like themselves.

FIG. 9—Oogonium from the lumen of the same ovary in process of division. Nuclear fission is complete and will be followed by that of the cytoplasm. Sperm heads lie in the peripheral cytoplasm, and do not hinder the division. The darkly staining spheres (blue with Mallory) probably represent material from the sperm heads in process of absorption.

FIG. 10—Older ovum from the lumen of the same ovary. Three other ova lie in contact with it, and it is also surrounded by free sperms. The ovum is larger, but contains fewer sperm heads, and the darkly staining zones in the cytoplasm probably represent absorption of sperms. From this stage onwards no further sperms pass into the cytoplasm of the growing ovum, although sperms are in free contact with it.

FIG. 11—Older ovum from lumen of ovary in March. Nearly all the sperm heads have been absorbed.

FIG. 12—Ovum of about the same size as the last which has absorbed all sperms and has become attached to the inner epithelium of the germinal ridge. Many sperms lie in the ovary lumen.

FIG. 13—Ovum at a slightly later stage from an ovary in March. The ovum has sunk into the germinal ridge and become overgrown by follicle cells with small nuclei. The edge of an intracellular space in the germinal ridge which is filled by sperm is cut in this section. (s.g.r.)

FIG. 14—Sperm head of average size drawn to the same scale as the ova here figured.

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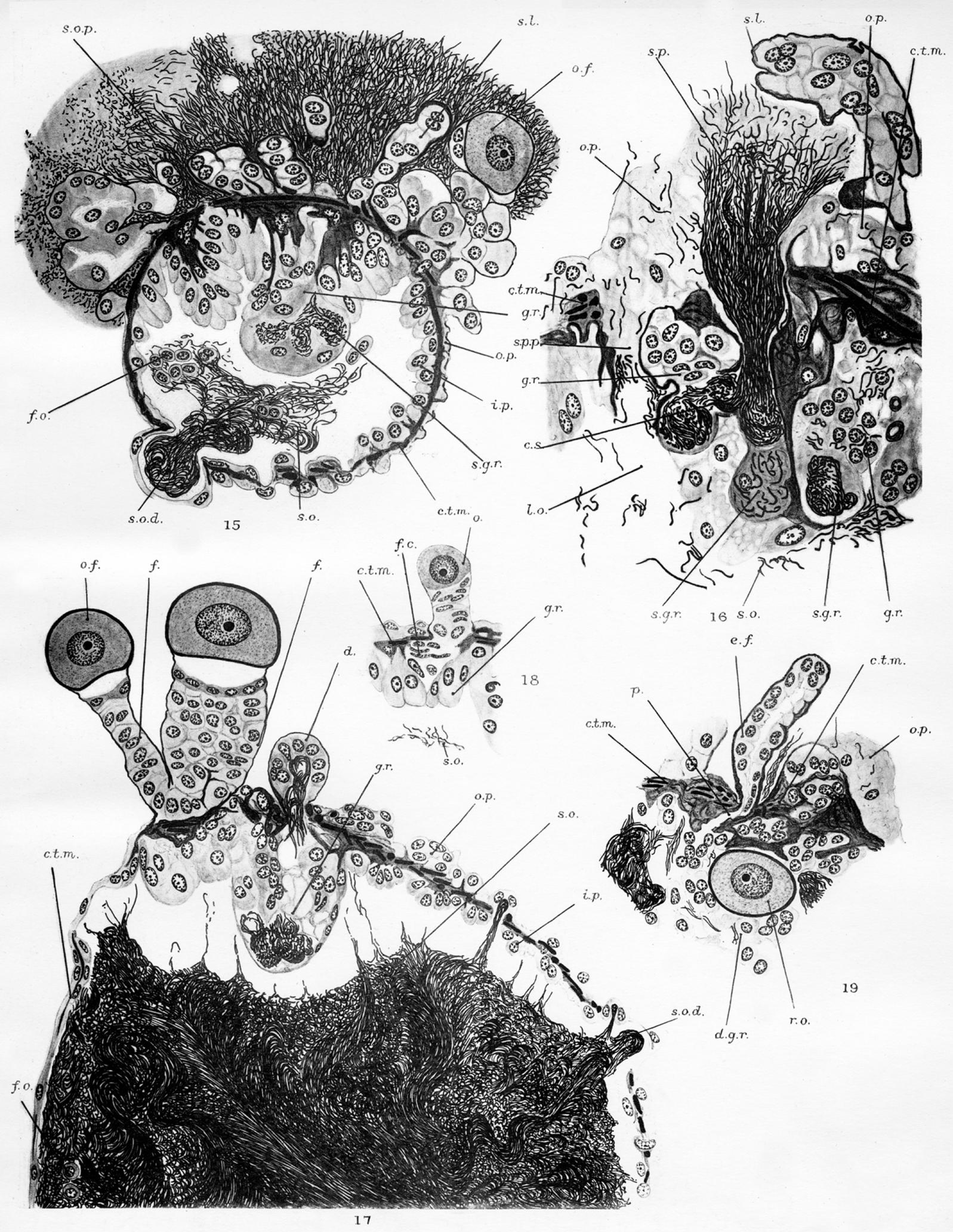

Plate 51

Sections of ovaries of *P. sedgwicki* showing the penetration of sperms into the ovarian lumen from the haemocoel, and the later development of the ova after they have sunk into the germinal ridge.

Fig. 15—Transverse section of ovary in July. A few very young ova and sperms lie in the lumen. The ovary is moderately contracted owing to the scarcity of solid contents; the germinal ridge projects into the lumen on one side, and externally project many sterile lobes (*s.l.*) and fertile follicles (*o.f.*). The cytoplasm of the outer epithelium of the ovary wall is swollen on the follicle side of the ovary, and is distended with a mass of sperm heads which will later pass into the ovarian lumen. × 290 approx.

Fig. 16—Transverse section of part of the ovary wall in March showing the penetration of sperms through the wall to reach the lumen. A clump of sperm heads (*s.p.*) have pushed in from the distended outer epithelium, and have partly penetrated through the connective tissue-muscle layer of the ovary, which is pushed inwards towards the germinal ridge. Another clump has already passed right through the wall to the lumen (*l.o.*) and has left an open passage (*s.p.p.*) through the connective tissue layer. Many intracellular sperms are seen in the germinal ridge, but the main mass of sperms lies in the ovary lumen outside the region figured. × 490 approx.

Fig. 17—Transverse section of half of an ovarian tube in May. Sperm penetration is now complete. No sperms lie in the outer epithelium or project from the haemocoelic side, and the lumen is distended with a great mass of sperms among which lie a few oogonia or ova. Some sperms lie in vacuoles of the germinal ridge. Two large ova project from the ovary on follicles. The stalks of the follicles are fully formed, flat and one cell thick, and differentiated distally into a darkly staining layer next to the ova. A membrane which stains blue with Mallory has been formed round the ova. Small diverticula of the ovary wall contain sperms from the lumen. × 290 approx.

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