STUDIES ON THE ONYCHOPHORA

VII. THE EARLY EMBRYONIC STAGES OF *PERIPATOPSIS*, AND SOME GENERAL CONSIDERATIONS CONCERNING THE MORPHOLOGY AND PHYLOGENY OF THE ARTHROPODA

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[Plates 31 to 41]

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There appear to be four different membranes round the eggs and embryos of the species of Onychophora at various stages of their development, and not two as previously supposed. The first membrane is left behind in the ovary, two further membranes occur in the stages of *Peripatopsis* here described, and an outer 'shell' is present in oviparous species. In *Peripatopsis* an inner cuticular membrane is absorbed at the end of segmentation, and an outer chitinous membrane persists until birth. These membranes influence the shape and volume of the embryos, and their properties and functions are described (pp. 489, 491, 527).

The uterine eggs and embryos of *Peripatopsis* undergo two sudden increases in size. In *P. sedgwicki*, *P. moseleyi*, *P. balfouri* and *P. capensis* the unsegmented egg swells and then remains constant in dimensions until the end of segmentation. In *P. sedgwicki*, *P. moseleyi* and *P. balfouri* the absorption of the inner membrane is followed by another dilatation which expands the blastodermic vesicle to form the large yolk sac. In *P. capensis* this dilatation does not take place and no large yolk sac is formed, but the membrane dilates as in the former species. In *P. balfouri* the dilatation of the embryo is followed by a sudden contraction which causes the early elimination of the yolk sac. The embryo then resembles the corresponding stage of *P. capensis*, in both cases floating within very large membranes until the embryos grow to fill them (pp. 489, 491, 501, 504, 512, 529, 537).

The unsegmented egg in *Peripatopsis* breaks up into a 'first blastomere' and a number of non-nucleated 'cytoplasmic spheres', all of which float freely in a watery fluid (pp. 496, 529).

Segmentation of the 'blastomere' results in a disk of blastomeres lying in a single layer against the egg membrane on one side. These cells give rise to the whole of the embryo and not to the ectoderm alone. The disk enlarges and becomes saddle-shaped (pp. 498, 530).

In *P. moseleyi* and *P. sedgwicki* the disk of blastomeres spreads all round the space within the membrane until its edges meet to form a continuous blastoderm. No blastopore is formed. The disintegrating remains of the cytoplasmic spheres lie in the internal space (p. 499).

In *P. capensis* and *P. balfouri* the edges of the disk of blastomeres curl away from the membrane, and 'large vacuolated cells' separate from these edges and pass to the concavity. A clear blastopore is

present in *P. capensis* and a transitory or virtual one in *P. balfouri*. In *P. capensis* the blastopore narrows to a slit and finally closes. The large vacuolated cells within form an endodermal lining to an archenteron, which may at first be partially filled by free vacuolated cells (p. 503). In *P. balfouri* the hemisphere of small blastomeres completely closes round the vacuolated cells; these form a solid mass, and then degenerate. Definitive endoderm arises later from a blastoporal area, as in *P. moseleyi* and *P. sedgwicki* (p. 509). The small embryo of *P. balfouri* then dilates while that of *P. capensis* does not. The gastrula of *P. capensis* thus corresponds with the hollow single-layered blastodermic vesicle of the other species. The large vacuolated cells form definitive endoderm in *P. capensis*, they are formed but degenerate in *P. balfouri*, and they do not occur in *P. moseleyi* and *P. sedgwicki* (pp. 530, 535, 536).

Evidence in support of the view that *Peripatopsis* is secondarily yolkless is provided by the details here presented concerning the form of the egg, segmentation, germ-layer formation, yolk sac, size changes, etc. (p. 535).

A germinal disk is formed upon a small part of the blastodermic vesicle in *P. moseleyi*, *P. sedgwicki* and *P. balfouri* just as it starts to dilate. The position of the disk varies specifically. It consists of a posterior thickening on which is situated a blastoporal area, and an anterior thickening from which is formed the mouth-anus. The further growth of the germinal disk and yolk sac is described for the several species. The blastoporal area gives rise to all the endoderm and mesoderm by immigration (pp. 505, 509, 510, 512, 513, 531, 536).

When immigration from the blastoporal area first starts in *P. capensis* and *P. balfouri* a 'giant cell' 30 μ in diameter sinks in. This cell is clearly glandular in *P. capensis*, passing droplets of secretion to its surroundings and possessing abundant mitochondria. In both species the cell disappears very soon. The disappearance in *P. balfouri* coincides with the sudden shrinkage of the yolk sac, which is suggestive of glandular activity of this cell. No such cell is formed in *P. moseleyi* or *P. sedgwicki* where the yolk sac remains large. This cell is possibly a specialization associated with the elimination of the yolk sac (pp. 512, 514).

The formation of the mouth-anus differs in the several species, but in all it is formed after the endoderm is established. In *P. capensis* the mouth-anus probably forms by a reopening of the closed blastopore. In *P. balfouri*, *P. moseleyi* and *P. sedgwicki* the mouth-anus arises *de novo* by a fusion of the ectoderm and endoderm to form the lips of this organ. Its size varies greatly in the different species. It may open widely and then divide to form mouth and anus, or it may not open until after this division. The mouth-anus does not give endoderm by invagination from the lips (pp. 513, 515, 518, 530, 531, 532, 545).

The formation of the mesoderm and of the mesodermal somites is described more fully than before, and with particular reference to the head end of the body. Details are given of the formation, growth and migrations of the somites, and of the origin of their coelomic cavities. The formation of somites is very uniform, and the process is simpler than that occurring in not only most Arthropoda but in the majority of Annelida as well. There is no trace of primary and secondary metamerism. The antennal somite is differentiated in a post-oral position and then migrates to a pre-oral one. The antennal somite is considered to be serially homologous with those following it. The origin of a coelomic cavity from one or several initial spaces is correlated with the size of the embryos (pp. 509, 519, 521, 546).

Germ cells do not arise in the endoderm, as previously supposed. They are differentiated at different stages in the several species, and arise from undifferentiated mesoderm or directly from the blastoporal area before any mesoderm has appeared. The subsequent history of the genital rudiment is described. A small proportion of its cells become incorporated into the walls of a variable number of mesodermal somites and the remainder degenerate (pp. 520, 525, 533).

Individual and bilateral variations are frequent, and many abnormal embryos have been found (pp. 497, 526).

No evidence has been obtained of the existence of a pre-antennal segment. It is suggested that no significance can be attached to the single case here recorded of asymmetrical mesoderm in front of the antennal somite, or to the embryo described by Evans for *Eoperipatus*, as the Onychophora have been shown to be subject to such extensive variations (pp. 525, 555).

The composition of the head in the Onychophora is considered. Support is given for the view that the antennal segment is the first segment of the body, and that it is the only one to become

pre-oral. Evidence is presented against the suggestion by Snodgrass that the antennal segment is part of an unsegmented acron (pp. 555, 556).

The data here presented indicate the existence of an underlying uniformity in ontogenetic processes displayed by the different species of Onychophora. Bouvier's suggested triphyletic evolution within the group is not supported. The range of variations found within the single genus *Peripatopsis* is remarkable. The methods of endoderm formation appear to show some of the responses of these species to an increasing amount of yolk in the egg which have persisted after a secondary reduction of the yolk. *P. capensis* appears to be the most advanced in the complete elimination of a large yolk sac, although it shows the more primitive method of endoderm formation; *P. sedgwicki* shows the least reduction of the yolk sac but has eliminated its primitive endoderm formation; *P. moseleyi* resembles *P. sedgwicki* except that the yolk sac is smaller; and *P. balfouri* has further reduced the yolk sac and shows an endoderm condition intermediate between the above extremes (pp. 536, 539).

The theory of head evolution in the Arthropoda presented by Snodgrass in criticized. The evidence for the view that the unsegmented acron includes the anterior region and the antennular and antennal segments in Crustacea, and the antennal segment in Myriapoda and Insecta, is considered to be unsound. It is suggested that this theory raises profound difficulties when applied to the Arthropoda. On the evidence now available, the Onychophora appear to show the lowest grade of arthropodan head evolution, three cephalic segments being present, only one of which has become pre-oral (pp. 555, 556).

The foundations of the theory of head segmentation in Annelida and Arthropoda put forward by Henry, Ferris and Hanke are criticized (p. 561).

The theories concerning primary and secondary metamerism are reviewed and a somewhat different interpretation of the facts is suggested. It is pointed out that primary and secondary metamerism must tend to appear in association with the possession of early-swimming larvae in which a premium is put on the early functioning of some parts of the body. The phenomenon is not considered to be primitive, as has been claimed. It is here suggested that the absence of a differentiation into primary and secondary segments in the Onychophora and certain Annelida is a primitive feature (p. 546).

The origin of many-segmented animals (p. 546) and the origin of metamerism are considered (p. 552). The suggestion by Snodgrass that metamerism arose in the ectoderm is not supported.

Other items arising out of Snodgrass's conception of arthropodan head structure are considered (p. 560).

A short review is presented concerning the blastopore, the mouth-anus of the Onychophora ('blastopore' of earlier workers other than Kennel), the mouth and anus, and the germinal disk in Annelida and Arthropoda. The two functions of the blastopore of the primitive annelidan type of development, (1) the putting in place of the endoderm (here by invagination), and (2) the formation of the mouth and anus by division, are completely dissociated in many Arthropoda and in many Onychophora, where a blastoporal area or blastopore are quite separate from the mouth and anus (p. 541).

The form of the germinal disk and yolk sac in the Onychophora and Malacostraca is shown to be directly comparable, and a criticism is made of Sollaud's interpretation of the latter (p. 545).

Introduction and acknowledgements

The investigation of which the results are here recorded was begun at the suggestion of Dr W. T. Calman. No species had been examined by adequate methods, and no full series of stages of any single type had previously been seen. The existing descriptions leave many gaps, they present many doubtful interpretations, and they suggest a diversity of morphogenetic processes within the group which does not in fact exist. Further data are required concerning the initiation and process of metamerism which will facilitate a further analysis of the morphology and phylogeny of the arthropod head, and recent contributions on metamerism based largely on segmented invertebrates need both confirmation and critical

examination. With these ends in view the present work has been undertaken. This contribution covers the earlier stages of development up to the establishment of the full number of body segments. Further work is in progress on the later stages and on other species.

Dr Calman took many steps to make this study possible, and it is a great pleasure to acknowledge his interest and encouragement throughout many years, and his kindness in reading the manuscript. I have to thank many friends in South Africa who contributed in helping to obtain adequate stocks of material, and in particular I am obliged to Dr R. F. Lawrence, Miss M. Johns, and Mr W. Saxton, whose collecting and general assistance were indefatigable. I am deeply obliged to Mr Saxton for the further supplies he sent me, as these animals provided many essential stages in the embryological series. The facilities provided by the Union and Castle Steam Shipping Company for the transport of the stock under cool conditions to England in 1933, and their many courtesies in carrying further supplies in 1934 are gratefully acknowledged.

This work has been carried out during a tenure of a Staff Fellowship, and latterly a Research Fellowship of Girton College, Cambridge, and various posts at King's College, Strand, London. Laboratory facilities have been provided by the Zoological Laboratory, Cambridge, the British Museum (Natural History), and by King's College. My thanks are recorded to all these institutions.

The earlier literature on onychophoran embryology is well known, and is considered here under the several headings in the description or the discussion.

The nomenclature followed is that of Bouvier (1905, 1907) and Clark (1913).

For convenience of reference to the figures similar developmental stages of one species are marked by a letter, *P. moseleyi* by *A*, *B*, etc., *P. sedgwicki* by *A'*, *B'*, etc., and *P. balfouri* by *a*, *b*, etc. Thus whole views, sections and reconstructions of the same stages of the same species bear the same letter. The planes of the sections figured are marked on either the whole views or reconstructions wherever the appropriate stages are shown.

The terms 'somite' and 'segment' are frequently used in a synonymous sense in the arthropodan literature. Here, as in the embryological accounts of the Crustacea (Manton 1928, 1934), the term 'somite' is used in a restricted sense meaning the mesodermal somite alone. The term 'body-segment' here refers to the whole unit, a series of which forms most of the body of the animal.

Material and methods

Most of the material for this study was collected in South Africa in April–July 1933, the expenses of the journey being defrayed in part by the Government Grants Committee of the Royal Society. Further supplies of animals were collected by Mr W. Saxton and sent by air and sea on several occasions. The animals were kept in glass tanks, containing decaying wood, at a suitable humidity. They fed on the micro-fauna and on small arthropods and pieces of sheep's liver. The stock of animals was maintained for embryological and other purposes in the Zoological Laboratory, Cambridge, for nearly four years. (For details of culture, life history, etc., see Manton (1938*c*).)

The material consisted of abundant specimens of *Peripatopsis balfouri*, *P. sedgwicki* and *P. moseleyi*, a few *P. capensis*, and some specimens of another species of *Peripatopsis* with sixteen legs from the neighbourhood of Grahamstown. This species (Manton 1938*b*, p. 478)

has not been described. Its embryos are of the *P. moseleyi* type (see pp. 491, 504, 506, 516, 517, 521, 522, text-figure 5 *a–d* and plate 33), and are quite unlike those of *Opisthopatus cinctipes* (also with sixteen legs) occurring in the nearby Uitenhage region, in which the embryos are at successive stages of development and are born at intervals over a long period (Purcell 1900) instead of being born at one period of the year as in *Peripatopsis*. In the following pages the dates are given when various embryological stages were fixed, and, unless otherwise stated, the material came from the main stock obtained in 1933. The reproductive cycle in captivity in the northern hemisphere may not correspond exactly with that prevailing in South Africa, but as the animals were kept at room temperature they did not experience fully the reversed summer and winter seasons of the northern hemisphere, and the breeding cycle of the female, occupying all months of the year, may not have been much altered.

Animals were killed with chloroform, and as each was opened the body fluid was collected in a capillary tube. The oviducts were removed and the embryos dissected out in a watch-glass containing parental body fluid under a binocular microscope. It is necessary at all stages to remove the outer chitinous membrane, which in early stages of some species is very thick and intractable, and this must be done without squashing or distorting the embryo, which in early stages has little coherence beyond the inner membrane. The transparency of the membranes enables damaged embryos to be detected and rejected at once. The partial or complete removal of this membrane in middle and late stages of development is easy, but during segmentation this is at times extremely difficult; it was done with a variety of needles in the body fluid either before fixation or immediately after immersion in the fixative. The inner membrane, present up to the end of segmentation, was perforated, but not removed, after fixation and before embedding, the perforations being made in different places in embryos of the same age. This can be done without distortion when the contents of the membrane are fixed, but cannot be done before fixation as the embryo is too fluid. Failure to perforate this membrane leads to great impregnation difficulties. A number of fixatives were employed, the most useful being formol bichromate and Duboscq-Brazil, in spite of the non-yolky nature of the material. The subsequent procedure differs little from that described for *Nebalia* (Manton 1934), alcohol being used for dehydration. Scale drawings were prepared with a binocular microscope of many embryos either living or just after fixation, so that structures here seen could be accurately correlated with the sections. Graphic reconstructions were made from the sections of a large series of embryos by the method employed for *Hemimysis*, etc., and described by Pantin (1946, p. 51). A clear and precise record of a large mass of material is thereby obtained for comparative purposes.

Migrations of the eggs and embryos and their changes of size

The youngest oogonia lie freely in the lumen of the paired tubular ovaries surrounded by sperms which they ingest for nutriment. They become attached to the wall of the ovary and sink into it, finally projecting externally at the tip of stalked follicles where they grow for a considerable time. At ovulation all or nearly all ova leave the follicles and pass back to the ovarian lumen and then on to the oviducts (for further details see Manton 1938*a*). Sheldon (1889*b*) inferred that this passage from follicles to oviducts must be rapid in *Peripatopsis capensis*, as she did not find any intermediate stages although several examina-

tions were made in April. This conclusion is supported by the present work on other species. Fertilization probably occurs in the ovarian lumen in *Peripatopsis* (Sedgwick 1885; Sheldon 1889*b*). No sperms have been seen to remain in the oviducts in this genus which lacks a receptaculum seminis, unlike *Peripatus novae-zealandiae*, where a receptaculum seminis is present and sperm do not lie in the ovary. The egg on regaining the ovarian lumen in *Peripatopsis* emerges from its membrane formed in the follicle (Manton 1938*a* and pp. 491, 527). The empty membrane is left in the ovarian lumen and later disappears. The naked egg is probably then fertilized, although the actual entry of the sperm has not been seen, and rapidly passes into the oviduct where it immediately becomes surrounded by two membranes before polar body formation and the fusion of the pronuclei is completed. The naked state of the egg must be of short duration, and may be necessary for fertilization, as the simple sperms have no mechanism for penetrating chitinous or cuticular membranes as have those of the Crustacea, and there appears to be no micropyle.

The ova in the ovarian lumen before fertilization are spherical or slightly elongated, 65 to 80μ in diameter in *P. sedgwicki*, the only species in which they have been seen, and with cytoplasm of even appearance lacking conspicuous inclusions. The eggs when ready for fertilization in other species are presumably larger where the follicular ova are larger. Bouvier (1904) records follicular ova of *P. balfouri* up to 370μ in size, and dimensions up to 380μ were found in material used for the present work. Ovaries of *P. moseleyi* were unfortunately not examined just before ovulation, but sizes up to 150μ were found at other times, while Bouvier noted some of 172μ; Sheldon (1889*b*) records a diameter of 260μ for *P. capensis*.

A rapid swelling, noted by Sheldon (1889*b*) for *P. capensis*, then follows. This apparently takes place by fluid absorption. The fertilized egg in the oviduct now takes on an elongated shape. Those of *P. moseleyi* amd *P. sedgwicki* are cylindrical with hemispherical ends (see figures 16 to 18, plate 32) as they are in *P. capensis* (Sedgwick 1885, figures 8 and 10, plate 31), but in *P. balfouri* the shape may be either cylindrical or more elliptical (figures 60 to 68, plate 37). This increase in size of up to three times the original length is greatest in *P. sedgwicki*, the species with the smallest eggs. The dimensions of the youngest eggs from the oviduct in *P. sedgwicki* were $260 \times 80\mu$, in *P. balfouri* $480 \times 220\mu$, in *P. moseleyi* $520 \times 160\mu$, and Sedgwick (1885) figures $600 \times 145\mu$ for *P. capensis*. The sizes in all species show considerable variation, which may be correlated in part with the size of the parents which do not reach maximum dimensions until the fourth year (Manton 1938*c*). *P. balfouri* is considerably smaller than the other species but it does not have the smallest eggs. When the egg rapidly swells by fluid absorption, it breaks up into separate spheres of protoplasm floating within the egg membrane (see p. 496 and figures 61 and 62, plate 37).

As is well known, the paired oviducts (or uteri) of *Peripatopsis* are simple tubes consisting of a short narrow upper section where the eggs are first received, and a wider and longer distal region opening to the exterior in which the embryos lie for about a year, each in a separate dilatation of the tube (figure 1, and Bouvier 1907, p. 99, text-figure 155). The structure of the upper oviduct is uniform throughout, there being no specialized glandular region. An inner epithelium of columnar cells is covered by a layer of connective tissue traversed by muscles. The thickness of both layers is dependent upon the degree of dilatation of the tube caused by the presence or absence of an embryo. The number of eggs

forming a brood varies specifically; it usually does not exceed about twenty-two in *P. capensis* and *P. balfouri*, sixteen in *P. sedgwicki* and twelve in *P. moseleyi*. The number also varies individually, and frequently almost exactly corresponds with the number of embryos forming the previous brood, for example, three, four, five and six pairs of eggs in the upper oviduct have been found repeatedly in specimens of *P. moseleyi* showing the same number of embryos respectively in the lower oviduct almost ready for birth.

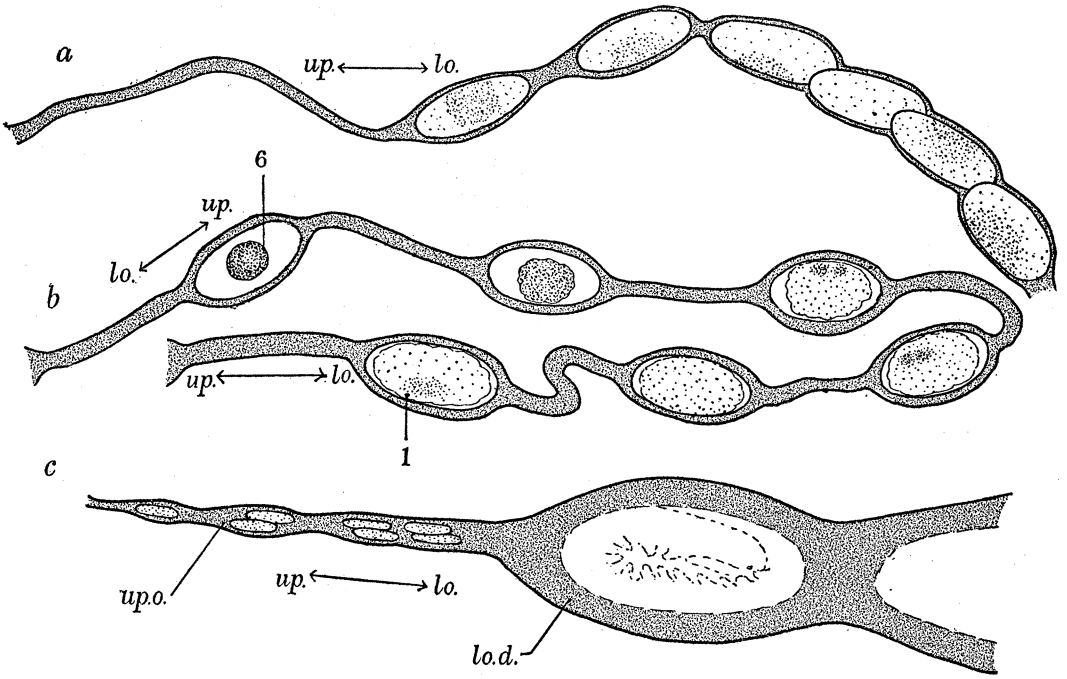

FIGURE 1. Diagram showing embryos in the oviduct. The internal margin of the duct is not shown, and the arrows indicate the directions of the upper (*up.*, internal) and lower (*lo.*, external) ends of the portions shown. *a. P. balfouri*, upper oviduct with early segmentation stages passing down towards their final positions in the lower oviduct. The areas appearing more opaque in life are stippled and represent the superficial blastomeres (see figures 63 to 66, plate 37). × 32. *b. P. balfouri*, lower oviduct with older embryos in their final positions. The uppermost embryo (1) nearly fills its membrane, and exhibits a transparent blastoderm with two opaque zones, the anterior and posterior thickenings of the germinal disk, which will form the mouth-anus and the blastoporal area respectively (see figure 72, plate 37). The succeeding embryos, each slightly more advanced than the one internal to it, show the contraction of the whole blastoderm to form a small opaque embryo (6) lying freely within the large space enclosed by the membrane (see figure 78, plate 38). × 32. *c. P. sedgwicki*, upper oviduct containing segmenting eggs which are passing down to the lower oviduct (*lo.d.*), which still contains embryos of the previous broods almost ready for birth. × 11.

The fertilized eggs in the upper oviduct segment as they are moved, presumably by peristalsis, towards their final positions in the lower oviduct (uterus). In the upper oviduct the segmenting eggs may lie close together in a row in *P. balfouri* (figure 1 *a*) and *P. moseleyi*, or the smaller eggs of *P. sedgwicki* may be crowded together into a double row (figure 1 *c*). One pecimen of *P. moseleyi* contained young embryos at 2- to 52-cell stages forming a single row from the ovarian origin to less than half-way along the upper oviduct, unborn embryos occupying the lower oviduct. By the time the embryos have traversed the whole of the upper oviduct

their development is much further advanced. On reaching the lower oviduct the embryos pass one by one into the places vacated by the birth of the previous brood, or in a female breeding for the first time, into sections of oviduct spaced well apart from each other. When they reach this position formation of somites has usually started; *P. moseleyi*, for example, may show the maximum dilatation of the yolk sac and the rudiments of six pairs of mesodermal somites. As noted by Purcell (1900) for *P. balfouri*, *P. capensis* and *P. leonina* 'the embryos of the same brood in one uterus form a series in successive stages of development, each one being slightly, although scarcely perceptibly, more advanced than the embryo on the side farthest from the external sexual opening In later stages this difference is no longer easy to observe'. In figure 1*b* the lower oviduct of *P. balfouri* is shown with a series of six embryos at clearly progressive stages of development comparable with those shown in figures 72 to 78, plates 37 and 38. Some ranges of development within single broods are noted on p. 495.

The developmental stages of most species of *Peripatopsis* after fertilization undergo two sudden increases in size. The enormous swelling leading to the non-coherent oviducal egg of fixed shape has been noted above. The volume within the egg membrane then remains approximately constant until the close of segmentation (see pp. 492, 499 and 500, and figures 8, 19 and 27, plates 31 to 33). After this another sudden increase in volume takes place by fluid absorption (p. 503). This is most conspicuous in *P. sedgwicki* and *P. moseleyi* and in the species shown in figure 5*a* to *d*. The yolk sac, which contains no yolk, swells greatly (figures 9 to 13, 28 to 36, 71, 72 and 76, plates 31, 33 and 37), and its walls are distended by fluid pressure, the component cells becoming greatly drawn out tangentially and their nuclei flattened (figures 48, 49, 106 to 109, *t.bl.*, plates 35 and 40). In *P. balfouri* a similar swelling takes place, but a sudden shrinkage of the yolk sac follows immediately (p. 512), leaving the dilated egg membrane far removed from the relatively small embryo consisting entirely of definitive embryonic tissue (figures 71 to 78, plates 37 and 38). In *P. capensis* no dilatation of the embryo occurs, and the segmentation stages contract to form a small embryo devoid of a yolk sac resembling *P. balfouri* after the absorption of this organ. The egg membrane, however, dilates in *P. capensis* (figure 2 (*m.s.* and *m.d.*)), as in the other species, and so the embryos in early stages float in a large fluid-filled space, as in *P. balfouri*. In all species subsequent growth is gradual, and the yolk sac in *P. moseleyi* and *P. sedgwicki* is progressively absorbed. Shortly before birth the outer membrane is ruptured, ecdysis starts, and the first peritrophic membrane containing uric acid crystals (Manton 1937) is passed out by the anus. The young *Peripatus* is then active and passes to the exterior, having completed its first ecdysis. A photograph by Holliday (1944) shows the actual birth of the young.

Membranes of the egg and embryo

The origin and fate of the membrane formed round the developing eggs on the ovarian follicles have been described (Manton 1938*a*). This membrane, the 'egg shell' of Sheldon (1889*b*) or 'vitelline membrane' of Dendy (1902), etc., reaches considerable thickness and stains blue with Mallory, but it does not persist into embryonic life, being left behind in the ovarian lumen prior to fertilization.

The fertilized egg in the upper oviduct is surrounded by two membranes, as noted by Sedgwick (1885) for *Peripatopsis capensis* and *P. balfouri* (see above). The *inner membrane* is

probably produced by the egg itself, as it forms a boundary of even thickness and regular form round the egg, which after its dilatation (see above and p. 496) is so aqueous as to have no independent coherence apart from this membrane. The formation of the inner membrane has not been seen. It is possibly a fertilization membrane, and it must arise in the ovarian lumen or in the extreme upper end of the oviduct. It persists for a short time only, roughly until the end of segmentation, and disappears before the embryo takes up its final position in the lower oviduct. The staining properties of the two membranes are usually distinct (see p. 494).

The *outer membrane* persists until birth, and is probably secreted by the wall of the upper oviduct. The process has not been fully observed and must be rapid. Normally, the lining epithelium of the upper oviduct is perfectly regular throughout, but sections through the ovarian ends of a pair of oviducts which had just received fertilized eggs show the free ends of the epithelial cells to be ragged and vacuolated, with every suggestion of a completed secretory activity, and opposite this epithelium lie fully shelled eggs. The outer membrane is often but not always thicker at the poles of the egg than elsewhere in very young stages, appearing to have flowed into the available space in each dilatation of the oviduct. The substance of the membrane may be slightly increased as the embryo grows, although dilatation of the embryo is accompanied by a marked reduction in its thickness. There is no evidence of any extensive secretory activity by the oviduct after the membrane has been laid down.

In *P. moseleyi* the two membranes are clearly separable, and the outer is very much thicker than the inner. During segmentation stages (stages *A* to *D*, plates 32 and 35) the outer membrane is about 20 to 50μ in thickness, being thinner at the sides than at the poles, and a lateral space or a terminal one also may be seen between the membranes in the living state. The outer membrane can easily be removed with needles leaving the inner one intact.

In *P. sedgwicki* the outer membrane is extremely thick (25 to 165μ*) in the few very early stages sectioned (Manton 1938*a*, text-figure 7, p. 430), but during later segmentation stages it is relatively thinner than it is in *P. moseleyi*, and the two membranes are then equal in thickness and closely coherent (figure 20, plate 32).

In *P. balfouri* the two membranes are often difficult to distinguish from one another, as the outer one is thinner than the inner at all stages, and is closely adherent to it. No space has been seen between them in life. In many preparations they are clearly separable (figures 100 to 102, plate 39) both morphologically and by staining, but in some the thin outer component is so closely applied and possibly shrunk on to the heavily staining inner membrane that an apparently single structure is seen in sections.

In *P. capensis* material has only been available after dilatation of the embryonic membranes has occurred. At the end of segmentation both membranes are thin, the outer is about 3 to 4μ thick and the inner one is very much thinner. They are often but not always adherent to one another. The outer membrane, unlike those of the three former species, shows in early stages a very thin inner border staining darkly with haematoxylin. This layer may tend to separate from the main membrane, and it disappears after mesoderm formation has begun.

* An erroneous thickness was given on p. 434, line 33 (Manton 1938*a*), 140 to 240μ should read 25 to 165μ.

The inner membrane disappears suddenly when the blastoderm is fully formed and gastrulation is about to start in *P. balfouri* (stages *j* to *k*), in *P. moseleyi* (stages *D* to *E*), and in *P. sedgwicki* (stages *B'* to *C'*, plates 31, 32, 33 and 37), leaving the embryo covered only by the outer membrane. This membrane and the enclosed embryo then dilate (see above). In *P. sedgwicki* the embryo always fills its membrane, which steadily increases as the yolk sac swells (figures 9 to 12, plate 31); the membrane then stretches less rapidly during the absorption of the yolk sac and further growth of the embryo.* In *P. moseleyi* the embryo similarly fills its membrane, but the stretching is less even. A great dilatation of the yolk sac occurs as soon as the inner membrane is absorbed (figures 27 to 32, plate 33*), and the embryo then remains at approximately the same size for a time while the definitive tissues increase and the yolk sac decreases. In *P. balfouri* the dilatation of the outer membrane and blastodermic vesicle is immediately followed by contraction of the yolk sac so that a small embryo comes to float in a large fluid-filled space bounded by the outer membrane (figures 77 and 78, plates 37 and 38). The membrane remains at approximately this size for a long period, during which the developing embryo gradually fills the internal space (stages *o–z*, plate 38).

The inner membrane in *P. capensis* persists a little later than in the above three species, disappearing when the mouth-anus is open and immigration from the blastoporal area has started, but unlike these species dilatation here occurs before the inner membrane is absorbed. Sedgwick (1885) records membrane lengths of 0·56 to 0·6 mm. during segmentation, but the youngest stage here examined, a late segmentation stage of about eighty cells (figure 120, plate 41), has an egg membrane just over 1 mm. in length. Other embryos a little older show membranes up to 1·5 mm. long. In all the shape remains the same, the sides being parallel (figure 2 (*m.d.*)), and some rigidity of form exists even after the inner membrane has disappeared. The absence of a large blastodermic vesicle in *P. capensis* (p. 491) results in the early embryos at all times floating in a large space within the outer membrane, as occurs in *P. balfouri* after yolk-sac absorption, and the membrane then remains constant in size until the embryo has grown to fill it.

In late embryonic stages of all species the outer membrane fits tightly round the ever-enlarging young, reaching sizes of approximately 3 to 4 mm. in the longer diameter in *P. balfouri*, 6 to 7 mm. in *P. moseleyi*, and intermediate sizes in *P. sedgwicki* and *P. capensis*. Just before birth the membrane is ruptured and passes out of the oviduct along with the young.

The stretching of the outer membrane in later embryonic stages may be due to pressure exerted by the embryo, but the early dilatation cannot be caused in this way, as it takes place in *P. capensis* independently from the embryonic tissue. Neither can it be a simple physical consequence of the removal of the inner and probably impermeable membrane as might be supposed in *P. moseleyi*, *P. sedgwicki* and *P. balfouri*, because in *P. capensis* the inner membrane shares in the dilatation before disappearing, and must thus be permeable. Further study of the membranes of *P. capensis* is desirable.

* The embryos figured for each species are from different parents of varying ages. Similar developmental stages show considerable variations in size. When an older stage is figured which is slightly smaller than a younger, as in figures 35 and 37, plate 33, this does not mean that a shrinkage normally occurs at this stage of development.

Properties

The *properties* of the two membranes differ markedly. The *inner membrane* stains red with Mallory and blue-black with iron haematoxylin. It has considerable rigidity and, except in *P. capensis*, stretches little if at all after it is fully formed. It maintains the definite specific shape (see p. 489) of the fertilized eggs and segmenting ova, with parallel sides and hemispherical poles. The interior of the embryos is too fluid in early stages to maintain any fixed shape, and the whole embryo when manipulated with needles in the living state, after the outer membrane has been removed, is very firm, almost like a bead. The membrane is not readily permeable to reagents, and has to be pricked to obtain satisfactory embedding. The staining reactions, rigidity and limited permeability of this membrane resemble the properties of the cuticular layer of arthropod integument, and the outer membrane of crustacean eggs.

The *outer membrane* stains blue with Mallory and usually remains unstained by iron haematoxylin, but some sections of *P. moseleyi*, *P. capensis* and of *P. sedgwicki* show the outer membrane darkly stained by iron haematoxylin during segmentation stages, and particularly after Duboscq-Brazil fixation. It has little rigidity and stretches readily, and appears to take on the form of the oviduct lumen around the embryo, having no fixed form of its own. When present alone it is flaccid and easily distorted, and the membrane with its contents is soft and flabby in the living state when handled by needles. The change in the 'feel' of the embryos after absorption of the inner membrane was particularly noticeable in *P. balfouri*, where great care became necessary to avoid squashing the living embryos. The cause of this change was not known at the time of preparation of the embryos for fixation. The outer membrane is freely permeable, and the embryo is nourished by materials passing through it. In its properties this membrane resembles the inner membrane surrounding many crustacean eggs.

Functions

The *function* of the inner cuticular embryonic membrane in *Peripatopsis* appears to be protective, as is the outer cuticular egg membrane in Crustacea (Mawson & Yonge 1938; Yonge 1938). But in *Peripatopsis* it also provides support for the early developmental stages, which are too aqueous to have any distinct form alone, and it is these young stages which must withstand transport down the upper oviducts on their way to their final positions in the lower oviducts. The completion of segmentation establishes a coherent embryo, and thus the need for support disappears. By this time the small amount of food material derived from the egg has been utilized, and the need to take in more raw materials must arise. These requirements are met at this stage by the solution of the cuticular membrane, so leaving only the permeable extensible outer chitinous membrane for the greater part of embryonic development. A survey of the embryonic membranes of the Onychophora is given on p. 527.

Segmentation

The most extensive series of segmentation stages has been obtained of *P. balfouri*, a smaller number of *P. moseleyi* have been examined and but few of *P. capensis* and of *P. sedgwicki*. The technical difficulties dependent upon the outer egg membrane, and the small number of early stages found in the material, resulted in regrettably few early stages

being successfully sectioned. Satisfactory preparations have been obtained of only four normal unsegmented ova, all of *P. balfouri*. The material obtained for *P. moseleyi* consisted of two families of 2- to 55-celled stages obtained on 5 and 26 March 1935 from animals captive for 3 months. All embryos were of small size, the second dilatation (pp. 491, 499 and 500) having not yet taken place, and the lower oviducts contained embryos nearly ready for birth. A third family obtained on 23 April 1934 showed a few undilated late segmentation stages, the other embryos being dilated and triploblastic, and some of the previous brood had already been born. Satisfactory sections were obtained of the 2-, 29-, 32-, 52- and 53-celled stages from one family, of the 18-, 20-, 21-, 40- and 55-celled stages from the second, and of the 130- to 140-celled stages from the third. For *P. sedgwicki* one very young family was obtained on 3 March 1936, but no satisfactory sections were obtained from it, and one family ranging from undilated late segmentation stages to dilated early gastrulation stages was obtained on 8 May 1934. Satisfactory sections were made of the 55-, 160-, 180-, 184- and 230-celled stages. Abundant material of *P. balfouri* from the 6-celled stage onwards has been obtained and sectioned. Early segmentation stages were found throughout May 1933 in animals freshly collected, and on the following 8 March and 6 April in captive animals. Late segmentation stages of *P. capensis* were obtained from 7 May to the middle of June 1933.

Segmentation in *P. sedgwicki* as far as it is known does not appear to differ markedly from that of *P. moseleyi*, but the process in *P. balfouri* and *P. capensis* shows conspicuous differences. The two types of cleavage appear to be differences of degree rather than of kind (see pp. 536, 539). The figures show embryos approximately as they appear in life, but details of nuclei have been added from sections. Dark zones represent vacant fluid-filled spaces and lighter areas the more opaque blastomeres and protoplasmic spheres. For further details of conventions see the legends.

The unsegmented egg

The ovarian egg shows a continuous cytoplasm of even granular appearance (Manton 1938*a*), but containing minute particles scattered throughout which have been identified as yolk by King (1926). Yolk formation has been followed by King, who noted that it appears before the mitochondria become distributed. After fertilization and dilatation of the egg (see p. 489) the cytoplasm no longer presents a uniform appearance, and the yolky material, possibly swollen by absorption of water (*y*, figures 60, 97 and 98, plates 37 and 39) separates from the protoplasmic portion (*p*).

The earliest stage taken from the oviduct is represented by two unsegmented ova of *P. balfouri* showing polar body formation. These ova occurred in a family ranging evenly from unsegmented ova up to 32- and 34-celled stages obtained on 23 May 1933. The eggs had presumably all been fertilized, as they were provided with typical membranes and did not appear abnormal in any way. The outer membrane could not be differentiated from the inner after formol bichromate fixation, but was clearly seen after treatment with Flemming without acetic acid. The gross features were seen in the living state, but the membrane buckled on embedding, so compressing its contents. In one of these eggs the two polar bodies have separated (figure 60, 97 and 98, plates 37 and 39). The egg cytoplasm

forms a thin irregular superficial layer all round the egg, thickening greatly on one side midway between the poles, the nucleus being located in this thickening. Fine strands of protoplasm pass inwards from the superficial layer, just as occurs in the yolky egged Crustacea (Manton 1928, text-figure 1 *a*), and disappear into a finely granular mass *y* which is mostly unstained by iron haematoxylin (figure 97, plate 39). This mass contains a number of heavily stained particles (figure 98, plate 39), and is presumably composed of food material which has separated from the cytoplasm, but unlike typical 'yolk' it is very soon dispersed and absorbed. The nucleus in this egg lacks a membrane and shows elongated chromosomes (figure 97, plate 39). One polar body (*p.b.* 1, figure 98, plate 39) contains degenerating chromatin masses, and a second polar body (*p.b.* 2) shows short paired chromosomes. This description of the early egg accords with the outline given by Sedgwick (1885).

All later stages differ from the above in that the egg contents become discontinuous. The transition from the coherent ovarian egg to that of the dilated egg in the oviduct (see below) has not been seen and needs further study. Within the embryonic membrane the continuous cytoplasmic zones break up, and separate spheres of cytoplasm of many sizes are formed which float freely in a watery fluid (figures 61 and 62, plate 37). Some of these spheres are composed of fairly uniform cytoplasm lacking inclusions, others are much vacuolated. In *P. balfouri* many large inclusions lie in the vacuolated spheres. One of the larger spheres of evenly granular cytoplasm contains the nucleus and constitutes the 'first blastomere', which segments and gives rise to the whole of the future embryo. Such a stage is shown in side view in figure 62, plate 37, and in section in figure 99, plate 39. No nuclear material is present in any other sphere of cytoplasm. The non-nucleated 'cytoplasmic spheres' do not give endoderm as supposed by Sedgwick (1886) and Purcell (1906) (see p. 530). All are absorbed or disappear by the end of segmentation. The form and fate of the cytoplasmic spheres and of the nucleated blastomere will be considered separately.

The 'cytoplasmic spheres'

The mass of the 'cytoplasmic spheres' of *P. moseleyi* is relatively less bulky than it is in the smaller egg of *P. balfouri*, and fewer but relatively larger spheres are formed. In a 2-cell stage (figure 16, plate 32) they lie towards the poles leaving the middle region vacant, but they may be situated in any position (see figure 18, plate 32). The larger spheres have a fairly regular margin and a very much vacuolated interior (figure 52, plate 35), and the smaller spheres are ragged in outline and vacuolated. No conspicuous cytoplasmic inclusions are present.

In *P. balfouri* the cytoplasmic spheres are more abundant. Purcell (1906) showed that some fixatives, including those used by Sedgwick, destroy the normal form of these spheres (which he as well as Sedgwick took to be endoderm). There is reason to believe that formol bichromate and other fixatives employed in the present work have given satisfactory results, as the reconstructions from sections correspond with the living appearance, although only gross features can be seen during life. In early stages the interior of the membrane is more or less filled with a loose mass of small cytoplasmic spheres and ragged bits of cytoplasm of many sizes which float freely in the fluid contents. They have no coherence one with another when liberated from the membrane. In some specimens viewed alive the

larger spheres are clearly seen, although many overlap forming a vague-looking mass (figures 61 and 62, plate 37), but in others the bulk of the spheres forms a more compact mass (figures 63 and 64, plate 37), which Sedgwick (1886) took to be non-nucleated endoderm (compare his figures 3 to 6, plate 31, 1885 with the above, and see p. 530). Sections show some of the larger spheres to resemble the even cytoplasm of a 'blastomere', while others are vacuolated to various degrees and contain many roughly spherical inclusions (figures 99 and 100, plate 39), much larger than those mentioned above in the coherent egg. These inclusions may stain black with iron haematoxylin, or they may appear brown or yellowish. With Mallory they appear orange, yellow or blue, or the central parts may stain yellow and the peripheral parts blue. It is probable that the inclusions consists of food material, since they are later absorbed. They are presumably derived from the mass *y* (figures 97 and 98, plate 39) of the early egg. As segmentation proceeds the cytoplasmic spheres disintegrate and disappear as in *P. moseleyi*. The inclusions become ingested and absorbed by the developing blastomeres (pp. 498, 500).

The 'blastomere'

Only two unsegmented ova have been found with discontinuous egg contents; they lack polar bodies and occur in a family of *P. balfouri*, slightly older than the unsegmented stages mentioned above, containing embryos up to the middle and end stages of segmentation besides normal young ready for birth. They were obtained on 6 April 1934. In one there is a single main sphere of cytoplasm containing a large vacuolated nucleus which floats in a position midway between the poles of the egg. It is shown in side view in figure 62, plate 37, and in section in figure 99, plate 39. The greater part of the interior of the membrane is filled by small cytoplasmic spheres such as are described above. The nucleated mass of cytoplasm lacks conspicuous inclusions and is for the most part denser and more uniform than the non-nucleated spheres. It is believed that this egg represents a normal stage of development, and that the cytoplasm containing the nucleus has rounded off from the rest of the egg contents seen in figure 60, plate 37 and figure 97, plate 39, the latter breaking up into cytoplasmic spheres containing food material.

Abnormal early stages

The other early stage in the family mentioned above is shown in figure 61, plate 37. The membrane encloses cytoplasmic spheres, but most of the chromatin lies in three spheres, a main sphere and one other being shown. The chromatin does not look normal, and it is believed that this is an abnormal egg which would have developed no further. Undoubtedly abnormal stages have been found in several families of *P. moseleyi* and *P. balfouri* in which nothing resembling a nucleus occurs. In these the membrane encloses a mass of protoplasmic spheres, some of which contain particles with the staining properties of chromatin. As many as five such specimens have been found in one family of *P. balfouri* otherwise consisting of normal embryos with all somites formed. The great gap in development between the advanced members of the brood and the stages in question also indicates the latter to be abnormalities. They are described here, as, unless a large series of stages is examined, specimens such as these showing 'good fixation' are most misleading.

First to sixth cleavages (2- to 64-cell stages) and formation of the 'saddle' of blastomeres

The further development of the nucleated sphere of cytoplasm within the egg (p. 497) will now be considered. Approximately equal division by cleavages at right angles to the surface gives rise to a disk or 'saddle' of blastomeres floating superficially at one side of the embryo immediately internal to the membrane. Some growth of the blastomeres takes place between the cleavages. From this disk the whole embryo is formed. An entire 'saddle' is seen in frontal view in figure 17, plate 32 and figures 63 to 68, plate 37, and a side view showing half a saddle in seen in figure 18, plate 32.

At the 2-cell stage (figures 16 and 51, plates 32 and 35, of *P. moseleyi*) the blastomeres are less thick than wide, and they press against each other and become thinner at their free margins. They lie midway between the poles of the embryo. Up to the 16-cell stage there may be great regularity in the arrangement of the blastomeres, which lie in transverse and longitudinal rows, as shown in figures 64 and 65, plate 37). Small gaps may occur where four cells approximate, unless shifting gives the stable position of three interfaces lying in contact (figures 17, 18 and 63 to 67, plates 32 and 37). Considerable variations occur in the orientation of the blastomeres about the egg axis (compare the 6- and 8-cell stages of *P. balfouri* in figures 63 and 64, plate 37). Beyond the 16-cell stage cleavages become less synchronous and the blastomeres become less regularly arranged, although few cells actually lose contact with their fellows (figures 17, 18, 66 and 67, plates 32 and 37). By the 64-cell stage the saddle of blastomeres extends round half to two-thirds of the circumference of the embryo and leaves the polar regions bare (figure 18, plate 32 shows a 53-celled stage of *P. moseleyi* in side view). The nucleus in early segmentation stages is either much lobed (*P. balfouri*, figures 100 to 102, plate 39), or in the form of many separate chromosomal vesicles (*P. moseleyi*, figure 51, plate 35), such as occur in early segmentation stages of many Annelida and Crustacea (Richards 1917). The nuclei become almost spherical by the 32-cell stage in *P. moseleyi* but are still considerably lobed at the 104-cell stage in *P. balfouri*.

The formation of the saddle of blastomeres is not influenced by the cytoplasmic spheres. The latter vary enormously in form and extent in different embryos; they float freely and appear to be in various stages of disintegration.

In *P. balfouri* the darkly staining inclusions originally present in the cytoplasmic spheres are ingested by the blastomeres of the saddle, where they become lodged in vacuoles. The marginal blastomeres are most active in this respect, and may be seen to contain many inclusions (figures 100 to 102, plate 39 of the 16- and 104-celled stages), but the inner parts of all the blastomeres also take up these inclusions to a lesser extent. Presumably a little cytoplasm may be taken up with them, or they may be ingested when the cytoplasmic spheres have disintegrated. Within the blastomeres, and also within the cytoplasmic spheres, the inclusions gradually lose their darkly staining properties, becoming paler and unstained by iron haematoxylin, and they eventually disappear. The changes in staining properties exhibited by these inclusions are reminiscent of the changes shown by the larger masses of yolk during development in Crustacea (Manton 1928, 1934), and it is reasonable to suggest that these inclusions are composed of reserve food material passed on from the egg to the developing embryo. All further nourishment is derived from the oviduct. The marginal

blastomeres ingesting most of the nutrient inclusions tend to become vacuolated and swollen (*sa.e.*, figures 100 to 102, plate 39), and beyond the 64-cell stage dilate considerably (figure 103, plate 39, and see below).

No stages of this age have been seen for *P. capensis*, but Sedgwick's work suggests that they correspond with those of *P. balfouri* but have fewer cytoplasmic inclusions.

Seventh to eighth cleavages (64- to 256-cell stages) and formation of a continuous blastoderm

The saddle of blastomeres described above gives rise to a continuous single-layered blastoderm surrounding a central space in *P. moseleyi* and *P. sedgwicki*. In *P. capensis* the saddle of blastomeres gives rise to a double-layered embryo with a blastopore, and *P. balfouri* shows an intermediate condition.

Late segmentation in Peripatopsis moseleyi *and* P. sedgwicki

In these species the formation of a blastodermic vesicle appears to be similar. By further mitoses the saddle of blastomeres (figure 18, plate 32) expands marginally until the edges meet. An intermediate stage has been found showing a cylinder of blastomeres round the middle of the embryo with open ends (figure 20, plate 32 shows such a stage for *P. sedgwicki* with fifty-five cells, which is clearly more advanced than the 53-celled saddle of *P. moseleyi*, figure 18, plate 32); later the poles close to give a continuous blastoderm. It is probable that variations exist in the exact manner of formation of the continuous blastoderm, but in the stages found nothing resembling a blastopore is formed. A continuous undifferentiated blastoderm covering the poles is present in 160-, 175-, 180- and 230-cell stages of *P. sedgwicki*, and in a 215-cell stage of *P. moseleyi* shown in figure 19, plate 32. The cells are thinner than at earlier stages and their nuclei are flattened tangentially. A transverse section of *P. moseleyi*, slightly older than that shown in figure 19, plate 32, is seen in figure 53, plate 35. The inner membrane has just been absorbed and the hollow blastoderm has started to dilate (see p. 491). Except in size, due to the onset of dilatation, the embryo resembles the stage shown in figure 19, plate 32, and within the undifferentiated blastoderm can be seen the last traces of the disintegrating cytoplasmic spheres. As soon as a complete blastoderm is formed the inner membrane disappears, and it is absent from the 160-cell stages onwards.

Late segmentation in Peripatopsis balfouri *and the formation and fate of the 'large vacuolated cells'*

It is probable that the early segmentation of *P. balfouri* and *P. capensis* is similar (see p. 501), but great differences occur at the end of this process. In both the formation of a continuous blastoderm takes place rather differently from the procedure in *P. moseleyi* and *P. sedgwicki*. After the 64-cell stage in *P. balfouri* the marginal blastomeres of the saddle (*sa.e.*), which contain most of the nutritive inclusions (*i.f.*) (see p. 498), dilate and become much vacuolated, increasing their diameter up to about four times. They bulge towards the concave side of the saddle, and almost or completely lose contact with its edges, and gradually pile into the concavity. A 104-cell stage is shown in figure 68, plate 37, in frontal view, and transverse sections across the middle and polar end are seen in figures 101 and 102, plate 39. The

saddle (*sa.*) comprises eighty-nine epithelial blastomeres; fifteen dilated marginal blastomeres (*l.v.c.*) have separated and shifted towards the concavity where they lie mainly towards the polar ends of the saddle. The remains of the non-nucleated cytoplasmic spheres (*c.s.*) are less abundant than at earlier stages (such as seen in figure 100, plate 39). The section through the middle of the saddle (figure 101, plate 39) on either side shows the slightly enlarged marginal blastomeres (*sa.e.*) rich in food inclusions, and the cytoplasmic spheres. The section through the polar edge of the saddle (figure 102, plate 39) shows two of the fifteen dilated cells (*l.v.c.*) which have separated and now lie among the non-nucleated cytoplasmic spheres (*c.s.*). (Reconstructions of such stages were necessary to determine the extent and positions of all nucleated and non-nucleated spheres of cytoplasm.)

The remaining cytoplasmic spheres and the food inclusions wherever they are situated become rapidly absorbed. The saddle of blastomeres extends, becoming thinner, and its edges curl in so forming a hollow hemisphere. More cells from the edges become swollen, vacuolated, and detached, and fill the concavity of the hemisphere. The incurled swollen edges of the hemisphere at first surround a large opening, but this narrows or becomes virtual as more vacuolated cells pass inwards (*B* of figure 103, plate 39). This opening or zone where the vacuolated cells are passing in clearly corresponds with the more conspicuous blastopore of *P. capensis* (see p. 503), but no archenteron is formed (cf. *P. capensis*) as the large vacuolated cells form a solid mass. (The definitive endoderm in *P. balfouri* arises later, see p. 512.) The whole embryo now forms a roughly spherical mass floating in the middle of the fluid-filled cavity of the membrane, having a very different external appearance on the two sides. A 113-cell stage is shown in plate 37, the epithelial hemisphere is seen in frontal view in figure 69, plate 37 and is composed of sixty-eight cells, fewer than in the 104-celled stage just described (figure 68, plate 37); the other side of the same embryo is shown in figure 70, plate 37, where the outermost of the forty-five huge vacuolated cells filling the hemisphere are visible. A section across the middle of this embryo is shown in figure 103, plate 39. The epithelium of the hemisphere (*s.a.*) is thinner than it was at the saddle stage (figure 101, plate 39) and the nuclei are flattened tangentially. The edges of the epithelium are continuous with some of the large vacuolated edge cells (*sa.e.*) bulging towards the concavity, and the latter is now filled by large free cells (*l.v.c.*). Each of these cells extends through many sections, and the nuclei of the nine free cells cut in the section shown in figure 103 have been projected on to the drawing from their positions in neighbouring sections. These vacuolated cells are clearly separable from the epithelial hemisphere with which they are in contact. The non-nucleated cytoplasmic spheres have all disappeared, having either completely disintegrated or been absorbed by the blastomeres, and no food inclusions (*f*, figures 99 to 102, plate 39) remain anywhere.

The epithelial hemisphere extends by mitoses and tangential expansion. Its edges spread over the large vacuolated cells lying on its concave side, and when they are covered a complete blastoderm results (figure 104, plate 39) and the blastopore is obliterated. As the blastoderm spreads round the vacuolated cells its nuclei become further apart, and often flattened owing to the lateral expansion of the blastomeres, but they are evenly distributed all round. There is no internal cavity, the whole forming what Purcell (1906) described as a solid gastrula. The vacuolated cells within become even more vacuolated, and tend to form a syncytial network, and they do not give rise to endoderm as supposed by Sedgwick

and Purcell. The disappearance of the inner membrane is now complete; this allows the intake of fluid from the oviduct through the permeable outer membrane, and both the embryo and its outer membrane start their rapid dilatation (figure 71, plate 37, and see p. 491). This stage is seen in longitudinal section in figure 104, plate 39. The internal vacuolated cells are greatly expanded and starting to disintegrate. The limits of each cell can no longer be determined, and their attenuated walls tend to break down where they are in contact with the blastoderm.

Dilatation of the blastoderm and outer membrane continues, and the latter attains a size which remains constant for a considerable period, until all the somites of the body have been established (plate 38). The blastoderm stretches until it fills the membrane, and its component cells become very attenuated. Within the dilated vesicle lies a disintegrating network formed from the large vacuolated cells. Portions of this are seen in figures 105 to 109, plate 40. Cytoplasmic strands (*n.*) containing a few nuclei (*d.n.v.*), many of which show clear signs of disintegration, are scattered through all or part of the vesicle. These strands come in contact with the developing germinal disk in some embryos, but can with care be clearly differentiated from the latter. In many embryos no strands lie near the germinal disk. The remains of the vacuolated cells have nothing to do with either endoderm or mesoderm formation, and they entirely disappear by the time the blastodermic vesicle condenses (figure 78, plate 38, see pp. 509 and 512). It is only in the earliest stages of gastrulation that the remains of the vacuolated cells are sufficiently numerous to confuse the appearance of the germinal disk in sections of some embryos.

The large vacuolated cells of transitory duration in *P. balfouri* represent the definitive endoderm of *P. capensis* (see below and p. 536).

Late segmentation in Peripatopsis capensis *and the formation of a gastrula*

Sedgwick (1885) gives two figures of early segmentation stages of *P. capensis* which correspond fairly closely with those of *P. balfouri*, when allowance is made for his fixation and interpretations (see p. 529), so that it is probable that *P. capensis* segments initially as do the other species. The earliest stage here examined, obtained on 7 May 1933, corresponds with stage *i* for *P. balfouri* (plates 37 and 39). A saddle or cap of about fifty epithelial blastomeres forms one side of the embryo, and its incurled edges are in contact with some of about twenty large vacuolated cells which have already piled into its concavity (figure 120, plate 41). The vacuolated cells contain small lightly staining inclusions and scanty cytoplasm, and otherwise resemble those of *P. balfouri* except for their lesser extent. The epithelial cells have much-vacuolated inner parts, a feature which is characteristic of *P. capensis*, and which persists for a considerable period (figures 120 to 123, plate 41). The non-nucleated cytoplasmic spheres have almost disappeared by this stage; a few remains are present near the embryo and among the vacuolated cells, but most of the large space within the egg membrane in devoid of formed elements.

A close series of stages has been obtained between the embryo described above and a gastrula with a closed blastopore. The cap of epithelial blastomeres becomes hemispherical, and the large vacuolated cells pass from its edges to the internal space. In some embryos the vacuolated cells project outwards as well as inwards as in figure 2*a*, but this is a temporary phenomenon, and soon all pass inwards and the edges of the hemisphere of

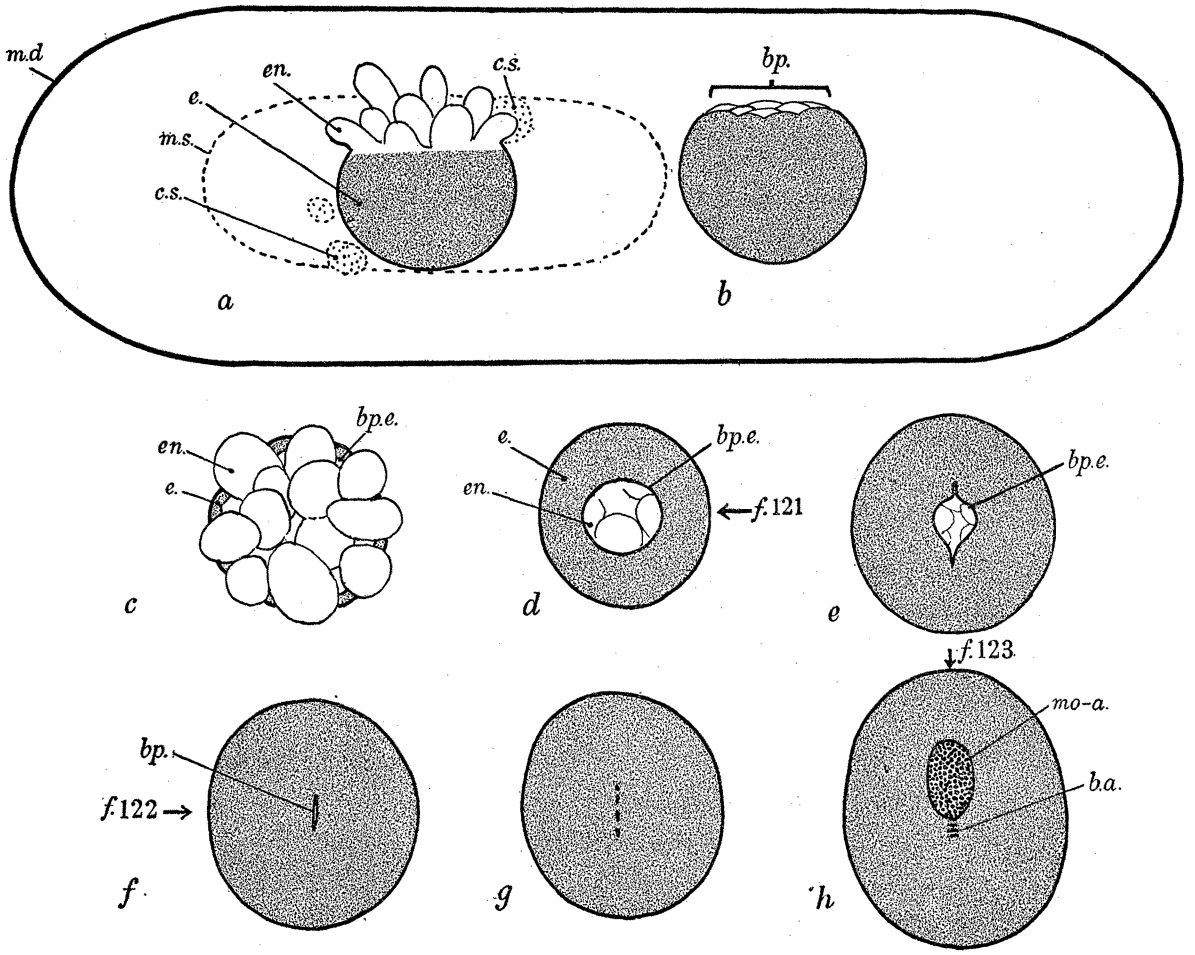

FIGURE 2. Diagrams of *P. capensis* showing the formation and closure of the blastopore. *a* and *b* in lateral view, *c* to *g* in blastoporal view, and *h* in ventral view. The ectoderm is mechanically stippled and the endoderm is white. The egg membrane (1·5 mm. long) which surrounded the embryo of *a* is shown (*m.d.*), but those from the other embryos are omitted. The dimensions of the undilated membrane (0·6 mm. long) surrounding slightly younger stages is shown by the dotted line (*m.s.*); the measurement of this stage is from Sedgwick (1885). *a*. An embryo a little older than that shown in figure 120, plate 41. A hemisphere of less than 100 small blastomeres (*e.* tinted) surrounds a small number of large vacuolated endodermal cells (white); eighteen of these lie within the hemisphere and nine are appended from its edges (*en.*). Diameter 230μ. *b*. Slightly older embryo from the same family in which the endodermal cells lie almost entirely within the ectodermal layer, and they can be seen filling the wide blastoporal opening (*bp.*). *c*. Blastoporal view of stage shown in *a*. *d*. Older stage than that shown in *b* in which the edges of the blastopore (*bp.e.*) have closed further over the endodermal cells. A section of this embryo is shown in figure 121, plate 41 along the plane indicated. Diameter at this stage 190 to 230μ. *e*. Older than the last. The blastopore is narrower, and its edges have come towards each other as shown. Diameter 260μ. *f*. Older than the last. The blastopore is a narrow short slit with the edges closely apposed. A section of this stage is shown in figure 122, plate 41 at the level indicated. Diameter 220μ. *g*. A slightly older stage in which the sides of the blastopore have united almost all along, the position of the blastopore is indicated histologically. Diameter 210μ. *h*. An older embryo with open mouth-anus (*mo.-a.*) and the first appearance of the immigration of mesoderm from the blastoporal area or primitive streak (*b.a.*). A section of this embryo is shown in figure 123, plate 41 along the plane indicated here and in figure 113. Width 250μ, length 280μ.

small blastomeres extend progressively over them (figure 2*b*). Internally the large vacuolated cells at once arrange themselves in an irregular single layer lying against the outer epithelium, and enclosing a central space which is more or less open to the exterior at the side of entry of the vacuolated cells. This archenteron may be a conspicuous cavity (*ar.*) or it may be partially obstructed by vacuolated cells (figure 121, plate 41).

The outer layer so formed gives the embryonic ectoderm, and the inner layer of vacuolated cells forms the definitive endoderm and does not degenerate as in *P. balfouri.* The zone of entry of the vacuolated cells is clearly a blastopore. At first it represents the full width of the embryo; then it narrows as shown in figure 2*b* and *d.* A few vacuolated cells lie freely in the archenteron of some embryos, the cavity of which may be considerably restricted by their presence (*f.en.*, figures 121 and 122, plate 41). In some embryos the blastopore is more or less plugged by loose vacuolated cells (figure 121, plate 41), but the lips of the blastopore can be recognized by their relation to the vacuolated cells; in others the blastopore is widely open. The blastopore narrows until a circular opening about 70 to 80μ in diameter is formed. The lips then come towards each other, starting from the two ends as shown in figure 2*e*. The closure continues until a very narrow slit about 30μ in length is formed in the embryo shown in figure 2*f* and figure 122, plate 41, ectoderm and endoderm are continuous at the lips of the blastopore, but are separate elsewhere. In the next stage obtained an association between the ectoderm and endoderm can be found on either side of a line about 70μ long which must represent the original blastopore, but almost everywhere a thin sheet of cytoplasm unites the ectoderm at the lips. The pale staining inclusions within the vacuolated endodermal cells have mostly disappeared by this stage, but many embryos show groups of very small darkly staining inclusions (figures 121 and 122, plate 41) which also shortly disappear.

A typical gastrula is thus formed, and its blastopore closes when the endodermal lining of the embryo has been established. The few vacuolated cells which lie freely in the archenteron in some embryos either degenerate or become incorporated into the endodermal layer. Very little, if any, endoderm is added during later stages (see pp. 512 and 513), the endodermal layer growing mitotically. During the changes described above an embryo is formed which is smaller than the diameter of the original saddle of blastomeres, a contrast to the other species where dilatation takes place. The embryo shown in figure 120, plate 41 is 308 $\times$ 200μ. The stages with a wide-open blastopore are about 230μ in diameter, and those with a small circular blastopore may shrink to 190μ. Embryos with closed or almost closed blastopores are 210 to 220μ. The further development of *P. capensis* is given on p. 513.

Formation of the germinal disk and first appearance of the blastoporal area in *Peripatopsis sedgwicki*, *P. moseleyi* and *P. balfouri*

Segmentation results in the formation of an elongated blastodermic vesicle filled with fluid (figures 19 and 71, plates 32 and 37) which then rapidly dilates in three of the above-described species and in the 16-legged species from Grahamstown, but not in *P. capensis* see figures 8 to 11, plate 31 for *P. sedgwicki*, figures 27 to 32, plate 33 for *P. moseleyi*, and figures 71 and 72, plate 37 for *P. balfouri*). Figure 8, plate 31 and figure 27, plate 33 show undilated stages of the same dimensions as the embryo in figure 19, plate 32. The form of the

thin blastoderm has been noted above (see *t.bl.* on figures 48, 49 and 105 to 109, plates 35 and 40). The nuclei are visible in life, but are not shown on the figures of whole embryos.

A germinal disk rapidly arises by a local division and aggregation of blastomeres, which lie close together and are consequently thicker, their nuclei soon elongating radially instead of tangentially. A small zone is thus formed, visible in life as an opaque spot, the edges of which fade into the more transparent surrounding blastoderm. The opacity is at first due to the thickening of the single-layered blastoderm, but later it represents thick tissue comprising internal cells also. The blastodermic vesicle now consists of (1) the germinal disk, and (2) the extra-embryonic portion which may now be termed a 'yolk sac', although it contains no yolk (see p. 537). The figures showing whole embryos (plates 31, 32, 33, 37 and 38) represent their living appearance by reflected light, the thicker opaque tissues appearing white and the thin transparent blastoderm appearing dark.

The germinal disk in *P. sedgwicki* and *P. moseleyi* starts to form just before dilatation of the embryo begins (figures 8 and 27, plates 31 and 33), but that of *P. balfouri* arises when the dilatation is completed (figure 72, plate 37). The germinal disk is usually terminal in position in *P. sedgwicki* (plate 31). In *P. moseleyi* it is usually lateral, appearing about midway between the poles of the blastodermic vesicle, and the long axis of the disk bears no fixed relationship to that of the vesicle, being situated in any direction in early stages (figures 28 to 34, plate 33). In these two species a few embryos have been found with germinal disks situated slightly laterally or more terminally respectively. The species shown in figure 5 *a–c* resembles *P. moseleyi* in the position of the germinal disk. In *P. balfouri* the germinal disk is perhaps more frequently lateral, but it arises in any situation, and, as in *P. moseleyi*, its longitudinal axis has no fixed orientation (figures 72, 76, 77 and 78, plates 37 and 38). In all species the germinal disk later becomes orientated so that its long axis coincides with that of the oviduct, but this is probably achieved by rotation of the embryo. A crumpled embryo of *P. sedgwicki* a little older than that shown by figure 13, plate 31, and an undistorted embryo of *P. moseleyi* a little older than that of figure 33, plate 33 were drawn by Bouvier (1905, text-figures 38 and 39) and illustrate the two positions of the germinal disk upon the yolk sac.

The germinal disk comprises two separate thickenings of the blastoderm. A posterior thickening arises first and gives rise to a blastoporal area from which all the endoderm and mesoderm is formed. It is present alone in figures 8 and 27, plates 31 and 33, where it is composed of about nine superficial and two internal cells. The formation of an anterior thickening quickly follows, and gives rise to the ectodermal part of the lips of the mouth-anus opening ('blastopore' of Sedgwick, Sheldon, etc.) and later to the mid-ventral ectoderm of the body.

In *P. balfouri* the anterior thickening (*a.g.*) is first detectable well in front of the posterior thickening (*p.g.*) from which it is separated by an expanse of thin blastoderm (figure 72, plate 37 and the reconstruction of the same embryo shown in figure 73, plate 37). Most of the anterior thickening forms the mouth-anus rudiment, and merges into the vesicle close to this organ (see section on figure 109, plate 40, through the embryo shown on figures 75 and 76, plate 37). Round the posterior thickening the change to the attenuated blastoderm of the vesicle is also abrupt (see sections in figures 105 and 108, plate 40 through the embryos shown in figures 72 to 76, plate 37).

In *P. moseleyi* and *P. sedgwicki* the anterior thickening is in contact with the posterior thickening from the start (see reconstructions in figures 21 and 40, plates 32 and 34, of the embryos shown in figures 9 and 29, plates 31 and 33, and figure 4, p. 517). The formation of the anterior thickening can be detected in sections before it is visible externally as in figures 29 and 40, plates 33 and 34. The mouth-anus rudiment first appears in the posterior part of the anterior thickening (figure 4) and spreads anteriorly, so making the thickening conspicuous as in figures 30 and 31, plate 33 (reconstructions of the same are shown in figures 41 and 42, plate 34) for *P. moseleyi*, and figures 9, 10, 21, 22 and 23, plates 31 and 32, for *P. sedgwicki*. The tissues of these larger species are relatively thicker than they are in *P. balfouri*, and the germinal disk may merge gradually and not abruptly into the thin blastoderm of the vesicle. Thus the germinal disk appears to spread farther out, and its edge (seen at the junction of *a.g.* and *t.bl.* in figure 4), lies much farther from the mouth-anus than it does in *P. balfouri* (figure 109, plate 40). In the reconstructions (figures 40 and 42, plate 34, and figures 21 and 22, plate 32) a pale grey area (*A* of the key on plate 34), representing thickened blastoderm, is shown extending far beyond the internal tissues and uniting the anterior and posterior thickenings, while the reconstructions of *P. balfouri* (figures 73 to 75, plate 37) show two very limited grey zones, and the anterior and posterior thickenings only just unite in stage *m*. There is some individual variation in the relative sizes of the two thickenings. The posterior is usually the larger, or the more conspicuous in *P. moseleyi* (figures 29 and 31, plate 33), but specimens have been found where they are about equal as in figure 30, plate 33 (see also the reconstructions of the same embryos in figures 40 to 42, plate 34). In *P. sedgwicki* and *P. balfouri* the two zones are about equal in size in early stages, and thus it is often impossible to identify the anterior and posterior ends before sectioning.

A blastoporal area arises on the anterior part of the posterior thickening. Its form and extent vary greatly in early stages, being either circular, oval, or narrow and elongated. From this area cells sink inwards to spread under the blastoderm. The inner germ layers arise entirely by immigration from this zone, and there is no trace of invagination. The parts of the blastoporal area from which endoderm cells pass in are shown by vertical hatching in the reconstructions, and the parts giving mesodermal cells by horizontal lines; where the two sets of lines overlap the immigrating cells supply both layers. In *P. moseleyi* cells forming the genital rudiment pass inwards with the earliest endodermal cells before any mesodermal immigration occurs (figure 39 onwards, plate 34). The genital rudiment in the other species arises from the mesoderm, and is shown by heavy dots in the reconstructions of all species (*D* in the key). The area shown by fine dots in the reconstructions of *P. balfouri* depicts a 'giant cell' which is also present in *P. capensis*, but is absent from the other species. For details of germ cells see pp. 519 and 525 and for the giant cell see p. 512.

When the mouth-anus rudiment is first established in *P. moseleyi* and *P. sedgwicki* its posterior extremity is only just separate from the anterior end of the blastoporal area (figures 21, 22 and 40 to 42, plates 32 and 34, and the longitudinal section in figure 54, plate 35 of a stage near to that of figure 21, plate 32). In *P. balfouri* the mouth-anus and the blastoporal area are at first widely separate, but when the blastodermic vesicle suddenly shrinks, the two are brought close together (figures 78 and 79, plate 38, and the longitudinal section of the same embryo shown in figure 110, plate 40), thus resembling in this respect the early

stages of *P. moseleyi*, *P. sedgwicki* and also *P. capensis*. Later development in all species results in the mouth-anus, or the anus, coming to lie a short distance in front of the blastoporal area, as seen in the reconstructions (figures 21 to 26, plate 32; figures 44 to 46, plate 34; figures 81, 84, 86, 89 and 91, plate 38; and the longitudinal sections in figures 111 and 112, plate 40).

Further development of the germinal disk and yolk sac in *Peripatopsis moseleyi*, *P. sedgwicki* and *P. balfouri*

The further development of the germinal disk will now be outlined separately for the three species named above. The limited material of the 16-legged species from Grahamstown (see p. 487) shown in figure 5 *a* to *d* indicates that it most closely resembles *P. moseleyi*.

Peripatopsis moseleyi

In *P. moseleyi* the thickened closed lips of the mouth-anus become visible externally as a transparent line or groove seen in figure 31, plate 33 (for details see p. 515), and the posterior thickening, consisting of superficial blastoderm, immigrating cells, mesoderm and endoderm has enlarged. The area lined by endoderm is at first visible as a slightly opaque zone extending beyond the germinal disk, seen clearly in figures 29 *en.* and 31, plate 33. By stage *J* endoderm lines the whole of the blastodermic vesicle (for further details see p. 510). The mouth-anus groove elongates and the posterior thickening spreads forwards on either side of the thickened lips, becoming almost continuous with them laterally, but never quite confluent (figures 32 and 43, plates 33 and 34). The arms of the now U-shaped posterior thickening form the germinal bands which establish all segments of the definitive embryo. They soon extend just anterior to the thickened lips of the mouth-anus rudiment, and then the bands bow outwards in their middle region. The first pair of somites (*s.* 1) become apposed just in front and close to the anterior end of the lips of the mouth-anus (stages *L* and *M*, plates 33 and 34). The succeeding somites lie farther from the lips, and posteriorly they converge towards the undifferentiated base of the U-shaped band which forms a transverse thickening, in the middle of which lies the blastoporal area (figures 33 and 44, plates 33 and 34). On either side of the thickened lips the ectoderm and endoderm become transformed into thin tissue as the bands move outwards, the epithelial cells becoming tangentially drawn out and more thinly distributed (*v.y.s.*, figure 44, plate 34). When the mouth and anus separate (stage *M* and see p. 518) the mid-ventral zone between them is similarly transformed, so giving with the lateral regions a thin ventral wall to the yolk sac bounded by the bands. This region becomes progressively larger as the bands elongate (stage *N*, plates 33 and 34).

The bands continue to bow outwards as they elongate, and they take a posteriorly directed curve in the hinder region as in stage *M* (plates 33 and 34), the mouth and anus being no farther apart at this stage than are the opposite ends of the continuous mouth-anus at stage *L* (plates 33 and 34). The blastoporal area lies a little behind the anus, and the germinal bands pass outwards and backwards before turning forwards. At stage *N*, where seventeen out of the twenty-three to twenty-seven somites* are differentiated, the mid-ventral region gradually stretches, and as it does so the bands become closer together

* The number of segments in *P. moseleyi* is twenty-three to twenty-seven and not a fixed number as in *P. sedgwicki* and *P. balfouri*.

ventrally and the anus and blastoporal area more posterior in position. The ventral wall of the yolk sac becomes narrower as the bands converge and is finally obliterated, a process well advanced in figure 38, plate 33. At the same time the elongating body becomes curled upon itself within the membrane (figures 37 and 38, plate 33). Two main flexures are present, but they do not occur at fixed levels.

The blastodermic vesicle thus bears the germinal bands upon its ventral side; the greater part forms a large 'yolk sac' lined with endoderm and filled with fluid situated on the anterior and dorsal side (*d.y.s.*), and an extensive ventral wall lies between the bands (*v.y.s.*) (figures 45 to 47, plate 34). The yolk sac separates the dorsal extremities of almost all pairs of somites at stage *N*, but it is not extensive between those of the posterior end of the body, where it is first absorbed. This progressive absorption of the yolk sac is shown in figures 37 and 38, plate 33. In figure 37 it has become much reduced behind the 8th somites both dorsally and ventrally, and anteriorly it is also smaller, while the lateral parts of the germinal bands have deepened at the expense of the yolk sac. In figure 38 the shrinking dorsal part of the yolk sac is largest anteriorly; posteriorly it forms a narrow tract between the somites, and a narrow remnant of the ventral wall of the yolk sac is still present. Later the yolk sac is completely absorbed as the germ bands unite both dorsally and ventrally.

There is considerable variation in the size and shape of different embryos of the same stage, and there is much bilateral asymmetry which is later eliminated. Reconstructions of an extensive series of embryos have been made showing the positions of the mesodermal somites, flexures, germ cells, etc., but only a few are here shown (plate 34). The asymmetries in figures 45 to 47, plate 34 are not artefacts, and an embryo with most of its somites established, which is bilaterally symmetrical, is exceptional, though it has been found. Somites from the 8th pair backwards tend to lie parallel to each other and to be separated by a small expanse of yolk sac, and the asymmetry from this level backwards is less than it is anteriorly. The varied orientation of the germinal disk upon the yolk sac is responsible for considerable asymmetry, as the anterior end of one side of the body may have greater freedom for expansion than the other. The extent of both the forward folding of the posterior part of the germ bands, and the onset and degree of coiling vary. A simple forward flexure of the posterior region may persist until all somites are formed, without any tendency to coil, and, alternatively, the coiling may start early, and turn to the right or the left. One embryo with only twenty-two pairs of somites and a still active blastoporal area shows a germinal band which between the levels of the mouth and anus measures about six times the length of the mid-ventral surface between these openings, and the bands make one and a half coils about the untwisted mid-ventral surface. All intermediate conditions have been found between these two extremes. As development progresses and the yolk sac becomes absorbed the bilateral symmetry of the body is improved, and is perfected when the mid-ventral surface elongates and the anus becomes terminal. The coiling of the embryo results in a compact body occupying a minimal space. The flexure of the embryo, however, is not at first controlled by the external pressure exerted by the membrane (see *P. balfouri*, p. 508 and *P. capensis*).

Peripatopsis sedgwicki

P. sedgwicki differs from *P. moseleyi* in the relative positions of the germinal disk and yolk sac (p. 504), but the growth of the germinal bands and yolk sac are essentially the same. The

terminal position of the germinal disk leads to the greater part of the yolk sac becoming anterior in position as shown in plate 31. The germinal bands and the ventral wall of the yolk sac arise as in *P. moseleyi*, but at an early stage a marked antero-posterior concavity is developed on the ventral surface as shown an figure 11, plate 31, the corresponding stage for *P. moseleyi* being much flatter. The body behind the 7th somite thus becomes much curled from the start, and the yolk sac here forms no more than a narrow dorsal and ventral tract at any time (figure 13, plate 31). The dorsal yolk sac may continue to dilate, reaching a maximum size in figure 13. Thereafter it becomes gradually absorbed and disappears as the bands unite to form the dorsal body wall. This happens first along most of the trunk region behind the head, leaving the remains of the yolk sac as a dorsal bladder attached to the head by a narrow neck (figures 14 and 15, plate 31). Finally it is completely absorbed. The ventral wall of the yolk sac disappears as in *P. moseleyi*, and the body becomes folded inside the membrane with increasing age, two main flexures being present (figure 14, plate 31). The asymmetry of the embryos is less extensive than in *P. moseleyi*.

Peripatopsis balfouri

In *P. balfouri* the early and sudden shrinkage of the blastodermic vesicle soon after its full dilatation, when the germinal disk is established but the germ bands not yet formed (figure 77, plate 37), greatly influences the form of the embryo. When this shrinkage is complete (figure 78, plate 38) a general thickening of ectodermal and endodermal tissues results, so that the germinal disk is only a little thicker than other parts of the embryo. Stage *r* (figures 83 and 84, plate 38) roughly corresponds with stage *K* (plates 33 and 34) of *P. moseleyi* in that there is an almost continuous germinal disk around the mouth and anus, the lips of these organs (or of the undivided mouth-anus) being almost continuous with the now U-shaped germinal band. By stage *s* (plate 38) the bands have elongated and they bow out, and thin-walled dorsal and ventral regions corresponding with the extra-embryonic yolk sac of *P. moseleyi* and *P. sedgwicki* are differentiated (*d.y.s.* and *v.y.s.*). This stage differs from *P. moseleyi* (stage *L*, plates 33 and 34) in the small size of the yolk sac, the earlier partition of mouth and anus, and the germ bands occupying a larger proportion of the surface of the embryo. The bands bow out a little farther as in stage *t* (plate 38), but on elongation of the mid-ventral region they approximate once more as in stages *u* to *z*. A slight ventral flexure is developed when eight to ten pairs of somites are formed (stage *v*), and then the hinder end of the bands becomes folded forwards as in stage *w*, where the eleventh and twelfth pairs of somites have appeared. Both anus and blastoporal area are carried forwards by the fold (stages *x* and *y*); in *P. sedgwicki* and *P. moseleyi* it is only the blastoporal area which is at first folded forwards to different extents in the two species (figures 12 and 35, plates 31 and 33), the anus remaining on the short unfolded region. The dorsal and ventral walls of the yolk sac are almost equal in size in *P. balfouri* (figures 93 to 96, plate 38). Both become narrower and are finally absorbed as in *P. sedgwicki* and *P. moseleyi*. The dimensions of the whole embryo gradually increase from stage *o* onwards, and by stage *y* the embryo fills the membrane once more. The flexure described above (figures 92 to 94, plate 38) is not caused by limitation of space, as it is initiated when the embryo is floating freely inside a relatively huge membrane (the membrane is shown in figures 78 and 95, plates 38, but is omitted from the other figures). The ventral surface gradually

elongates until the anus becomes terminal (figures 95, 96), but this is completed only after germ-layer formation has ceased and all somites are formed, as in *P. moseleyi*. The embryo becomes coiled inside the membrane with two main flexures as in the other species.

First stages of immigration in *P. moseleyi*, *P. sedgwicki* and *P. balfouri*, and general remarks upon this process

The endoderm, the mesoderm and the germ cells, when they are differentiated early, arise by immigration from a blastoporal area. The establishment of these layers occurs in essentially the same manner in all three species, but the appearance of the early stages of this process is influenced by the precocious development of germ cells in *P. moseleyi*, and by the formation of a 'giant cell' and the general thinness of the tissues in *P. balfouri*. In *P. balfouri* the remains of the network formed from the large vacuolated cells has not entirely disintegrated before immigration starts, but the nuclei of the latter can be distinguished from those of the germinal disk, even in embryos where the two structures come in contact (see p. 512).

As in other arthropods showing gastrulation by immigration, there is little difficulty in following the process where fixation is good and a complete series of stages is obtained. Outside the blastoporal area the blastoderm is even, the component cells are rectangular, their nuclei lying at all depths from the surface, but the exposed cell boundaries are evenly situated. If internal tissue is present this is sharply demarcated from the overlying ectoderm, even if there is no actual gap between the two. Within the blastoporal area none of these features are found. The immigrating cells lie at all levels; they are spindle-shaped, and there are clear gaps left at the outer surface where cells are in progress of sinking in. Internally there is no demarcation between the irregular immigrating cells of the blastoporal area and the internal cells with which they are in contact. All these features are seen with considerable clarity in the transverse and sagittal sections shown in figures 107 to 112, plate 40 for *P. balfouri*, figures 49, 50 and 59, plates 35 and 36 for *P. moseleyi*, and figure 54, plate 35 for *P. sedgwicki*, the blastoporal area being indicated by the bracket (*b.a.*). Mesodermal immigration is clearly seen in figures 54, 59 and 110 to 112, plates 35, 36 and 40, and endodermal immigration in figures 49, 50 and 54, plate 35. If, as in figure 54, the anterior limit of the blastoporal area is not apparent with diagrammatic clarity, it must be remembered that only one level can be shown here, and examination of neighbouring levels reveals no doubt as to the extent of the blastoporal area and its relationship to other things such as the developing mouth-anus opening.

The earliest cells to become internal are situated below the posterior thickening of the germinal disk (see below). Stages with only one internal cell onwards have been obtained. Where very few internal cells are present (as in the longitudinal section (figure 48, plate 35) for *P. moseleyi* with two internal cells, and in the transverse section (figure 105, plate 40) for *P. balfouri* with four), it is not possible to ascertain their exact points of immigration, but as soon as the process is a little more advanced, immigration is seen to be restricted to a narrow median zone, with regular blastoderm on either side (see transverse sections of *P. balfouri*, figure 108, plate 40 and of *P. moseleyi*, figure 59, plate 36, and longitudinal section in figure 58, plate 36 of *P. moseleyi*, figure 46, plate 34). At first the blastoporal

area is round or oval in *P. moseleyi* and *P. sedgwicki* (see reconstructions, figures 39 to 41, plate 34 and figures 21 to 23, plate 32), but in *P. balfouri* it is narrow and elongated (figure 75, plate 37). Later it becomes longer and narrower in all species, but may shorten and widen in later stages when mesoderm only is being formed. There is, however, variation in the shape and extent of the blastoporal area at similar stages; for example, the reconstructions of *P. moseleyi*, figures 41 and 42, plate 34, show a long and narrow zone in one and an almost circular one in the other. Where immigration is taking place rapidly and is restricted to a narrow elongated zone, a 'primitive groove' is apparent in transverse sections as noted by Sedgwick for *P. capensis*, and tends to be formed in the middle or anterior part of an elongated blastoporal area or 'primitive streak'. The groove is much shallower in *P. moseleyi*, *P. sedgwicki* and *P. balfouri* than it is in *P. capensis*. The blastoporal area* is maintained until mesoderm sufficient for the formation of all somites has been established, and then it disappears. Endodermal immigration ceases much earlier (see pp. 511 and 513). In the embryo of *P. moseleyi*, shown in figure 47, plate 34, the full number of twenty-seven somites has been formed, the cavity of the last pair is appearing, and the blastoporal area has just ceased to function. Figure 96, plate 38 shows the corresponding stage for *P. balfouri*.

The ultimate origin of the immigrating cells cannot be determined by the available methods for studying a viviparous embryo such as that of *Peripatopsis*. Mitoses in the blastoporal area are frequent, but they also occur in the general blastoderm. It is not unlikely that cells migrate towards the blastoporal area, and that mesodermal and endodermal cells are determined in an external position, perhaps some distance from the blastoporal area, and long before they are incorporated into the latter and pass inwards.

Early stages of endoderm formation in *Peripatopsis moseleyi* and *P. sedgwicki*

In *P. moseleyi* and *P. sedgwicki* the earliest detectable germinal disk comprises about six superficial cells forming the posterior thickening. By the time that this thickening contains about nine superficial cells one or two have slipped inwards and lie below the outer layer. The first cell to become internal swells when it reaches this position and the cytoplasm becomes vacuolated. The vacuoles either appear empty or filled with a substance staining pale blue with Mallory and remaining unstained by iron haematoxylin. The nucleus often becomes distorted as it lies in the irregular cytoplasmic boundaries of the vacuoles. An embryo from the same family of *P. sedgwicki*, as seen in figure 8, plate 31, but slightly younger, shows one such internal cell, and a *P. moseleyi* (figure 27, plate 33) with two internal cells is seen in section in figure 48, plate 35. One of these cells (*en.*) exhibits the features described, and the other is not yet differentiated cytologically from the superficial cells. Sections of early gastrulation of *P. sedgwicki* are not figured, as they are so similar to those of *P. moseleyi*. The first few cells to pass in develop similar characteristics and become endoderm. They vary greatly in size, some swelling to the full diameter of the germinal disk, and others undergo mitotic division soon after becoming internal. They spread outwards and away from the blastoporal area, becoming progressively more vacuolated, and forming a

* The term 'blastoporal area' is used here in preference to 'primitive streak', as it implies no limitation of function to the formation of mesoderm alone.

loose single layer of cells below the superficial tissues (figures 49, 50 and 54, plate 35). They appear very like the recently immigrated endodermal cells of a yolky arthropod such as *Hemimysis* or *Nebalia* (Manton 1928, 1934) in which the yolk vacuoles have been replaced by the vacuoles described above. This process of endoderm formation continues after mesoderm immigration has become active, and is then localized at the extreme anterior end of the blastoporal area, little if any mesoderm being formed here (see figure 24, plate 32 for *P. sedgwicki* and figures 40 to 45, plate 34 for *P. moseleyi*). The sheet of endodermal cells spreads rapidly (figures 28 to 32, plate 33) and extends beyond the germinal disk in an ever-widening circle, visible in life as an area slightly more opaque than the thin blastoderm of the vesicle, and shown by the pale area *en.* in figures 29 and 30, plate 33 of *P. moseleyi*. The endoderm spreads by mitoses and immigration until it lines the whole of the blastodermic vesicle, a process completed at stage *J* for *P. moseleyi* and *E'* for *P. sedgwicki*. Endoderm immigration is thereafter much slower, and when the posterior part of the definitive embryo folds forwards, it ceases. This occurs in *P. sedgwicki* when eight pairs of somites have been formed (figures 11 and 26, plates 31 and 32), and in *P. moseleyi* when about seventeen pairs have been established; however, it varies a little in the latter species, and embryos with sixteen somites (stage *M*) show some endoderm formation, it is doubtfully being formed in some with eighteen somites, and could not be detected in the embryo shown in figures 46, plate 34 with nineteen somites or in older stages.

The immigration of the endoderm, except in the earliest stages, is less easy to observe than the more extensive mesodermal immigration. When the mesodermal bands are forming (figures 22 to 25, 43 and 44, plates 32 and 34) a thick mass of mesodermal cells separates the endoderm from the greater part of the blastoporal area. The endoderm forms a continuous epithelium distinct from the superficial tissues everywhere except at the extreme anterior end of the blastoporal area, in front of the mesodermal immigration, and here the endoderm merges into the blastoderm, as seen in the longitudinal section in figure 54, plate 35 of *P. sedgwicki*. When endodermal immigration ceases, the whole of the endoderm forms an epithelium which is clearly separable from the superficial tissues of the blastoporal area as well as elsewhere, and in all regions it appears as distinct as it does in the transverse section of *P. moseleyi* in figure 59 *en.*, plate 36. The point of endodermal origin is at first close behind the developing mouth-anus (*l.*, figure 54, plate 35), but later it becomes separated from it (or from the anus) by a short distance (see p. 505).

The endodermal cells below the germinal disk in *P. moseleyi* remain vacuolated in most regions for a considerable period and their cytoplasm is scanty (figures 57 to 59, plate 36), but they form a more uniform epithelium with increasing age, the cells lying closer together (compare figures 50 and 58, plates 35 and 36). In *P. sedgwicki* the endodermal cells below the germinal disk may soon become less vacuolated, but the cytoplasm is not materially increased in amount, and so this layer appears attenuated as in figure 54, plate 35. There is no significant difference between the endoderm of these two species in older stages. Below the extra-embryonic ectoderm of the yolk sac the endodermal cells are much drawn out, vacuolated and thin, often with very scanty cytoplasm.

Further increase in the endodermal layer occurs only by mitosis. This leads to a thickening by closer approximation of the cells in the regions of the definitive embryo. By this means is formed an endodermal lining of the posterior part of the body, which first appears as

a diverticulum of the yolk sac. Many mitoses are found in this actively growing epithelium close to the base of the mesodermal bands, internal and near to the blastoporal area. As the yolk sac shrinks its endodermal lining becomes absorbed into the uniform epithelium of the gut by the approximation of its cells.

Formation of the endoderm and the 'giant cell' in *Peripatopsis balfouri*, and the sudden contraction of the blastodermic vesicle

As described above (pp. 501 and 509) the remains of the 'large vacuolated cells' persist until the onset of endoderm and mesoderm formation. They may come in close contact with the germinal disk of some embryos, and can be seen in the sections in figures 105 to 109, plate 40 (*n.* and *d.n.v.*), but they have no influence on gastrulation and give no adult tissue. The posterior thickening of the germinal disk at its earliest appearance is composed of a 'giant cell' with a large, lobed nucleus, dispersed chromatin, and uniform pale staining cytoplasm, surrounded closely by a few undifferentiated cells (*g.c.* and *u.c.*, figure 105, plate 40). This section passes through the plane indicated on the whole view and reconstruction, figures 72 and 73, plate 37, and cuts through part of the giant cell (dotted area in figure 73) and a few of the fourteen undifferentiated cells composing the posterior thickening. Some of the latter are in mitosis and some are just internal. The edge of this zone is sharply demarcated from the surrounding attenuated blastoderm. The transverse section in figure 106, plate 40 and the reconstruction in figure 74, plate 37 show a later stage. The giant cell is larger, the surrounding cells are more numerous, and the inner ones are becoming endoderm. A few of these now lie internal to the giant cell where they establish a connected layer, and others lying peripherally and within the outer blastoderm are becoming vacuolated (*en.*) and have spread beyond the posterior thickening, as indicated by the dotted line on the reconstruction in figure 74. The number of cells in the posterior thickening rapidly increases (figure 107, plate 40), and the endodermal layer spreads outwards in all directions, the margins being visible on either side of the figure. The multiplying undifferentiated cells add to the endodermal layer and spread superficially over the giant cell which thus becomes internal. By stage *m* (plates 37 and 40) the circular sheet of endoderm has spread far beyond both anterior and posterior thickenings (figures 75 and 108), the giant cell has become more deeply situated but is otherwise unchanged (only a small portion of it is cut in the section drawn in figure 108). The smaller cells composing the posterior thickening are now clearly organized into an outer thick blastoderm interrupted mid-ventrally by an elongated blastoporal area (*b.a.*), and from the latter cells pass in to become endoderm or mesoderm. The mesodermal cells (*me.*), first detectable at this stage, are few and lie between the thickened blastoderm and the endoderm; they cannot be clearly differentiated from the future endodermal cells, but resemble in position and appearance the mesodermal cells of later stages (figures 110 to 112, plate 40). Further immigration leads to rapid spread of the endodermal layer. It never lines the large blastodermic vesicle, as it does in *P. moseleyi* and *P. sedgwicki*, as a sudden reduction in the tension on the walls of the vesicle at stage *n* (mentioned above, pp. 491, 493 and 508) leads to a condensation of that part of the vesicle lined by endoderm (*bl.e.*) to form a small sphere on to which is appended the shrinking remains of the single layered part of the vesicle (*bl.s.*, figure 77, plate 37). The completion of the

condensation of the embryo (figure 78, plate 38) results in the appearance of a double-layered sphere, vacuolated endoderm being present throughout. The nuclei of both ectoderm and endoderm are now closer together and the cells are deeper as a result of the general shrinkage (see longitudinal section, figure 110, plate 40).

The 'giant cell' described above entirely disappears during this condensation of the embryo, leaving no trace. It is possible that the two events are correlated, neither giant cell nor shrinkage of the vesicle occurs in *P. moseleyi* or *P. sedgwicki*, and the giant cell has never been found after the sudden condensation of the embryo. No further facts concerning this cell have been obtained. Possibly it is a gland cell which influences the osmotic relations of the embryo, and on discharge causes a reduction in the osmotic pressure within the vesicle and consequently the shrinkage. The mouth-anus opens just after the shrinkage (see p. 517), but appears to be closed until then by a fine sheet of protoplasm, and so its opening cannot cause the reduction in internal pressure.

Thus the condensed embryo, lacking germ bands, is lined throughout by endoderm. This layer remains distinct from the superficial tissues at all later stages (figures 110 to 112, plate 40). No further additions to it by immigration from the blastoporal area could be found, and further growth takes place by mitoses; it is possible that a small amount of immigration too slow to be detected may take place from the anterior end of the blastoporal area, but no major immigration of endoderm occurs. Endoderm formation thus ceases much earlier in *P. balfouri* than in *P. moseleyi* and *P. sedgwicki*.

Beyond stage *o* the endodermal cells become closer together and deeper as they increase in numbers (figures 110 to 112). A surplus of cells piles up in the dorsal region of the middle part of the body (figures 111 and 112). Here the already much vacuolated cytoplasm disintegrates and the nuclei degenerate. All stages in the process have been found, some of the nuclei nearest to the gut lumen in figure 111 are almost free from their disorganizing cytoplasm and show stages of chromatin degeneration (*d.n.*). (For the evidence against the supposed invagination of endoderm from the lips of the mouth-anus (blastopore of earlier workers other than Kennel) see p. 518.)

Further development in *Peripatopsis capensis*

Sedgwick assumed that the open blastopore (see p. 503) of *P. capensis* gave rise directly to the large opening which divides to form the mouth and anus, but in fact he had no stages between one with an open blastopore 204μ in diameter and another of $448 \times 480\mu$ with a large mouth-anus (his figures 19 to 22 on plate 31, 1885, are not drawn to the same scale). This gap has been reduced, but not entirely filled, by the present work, as no stage between one with an almost closed blastopore 210μ in diameter and another of $257 \times 286\mu$ with a wide open mouth-anus has been found (figure 2*g* and *h*). The latter is younger than the corresponding stage obtained by Sedgwick. Thus there is no direct evidence as to how the mouth-anus arises. It is probable that it is formed by a reopening of the blastopore, and if this is so, *P. capensis* differs from the three species with a dilated blastodermic vesicle here described (p. 515).

The mouth-anus in the youngest stage obtained is $45 \times 70\mu$ in size, with ectoderm and endoderm continuous at the extreme edges of the lips which do not protrude (figures 113 and

123, plate 41). The free endodermal cells of some earlier stages no longer lie in the enteron in this or in similar aged embryos, and the enteric space is bounded by a regular layer of vacuolated endodermal cells. There is no evidence of endoderm arising by invagination from the lips of the mouth-anus (see p. 518). The endoderm present at this stage is derived in the manner already described by immigration from the edges of an open blastopore. Behind the open mouth-anus immigration from a rudimentary blastoporal area lying in the

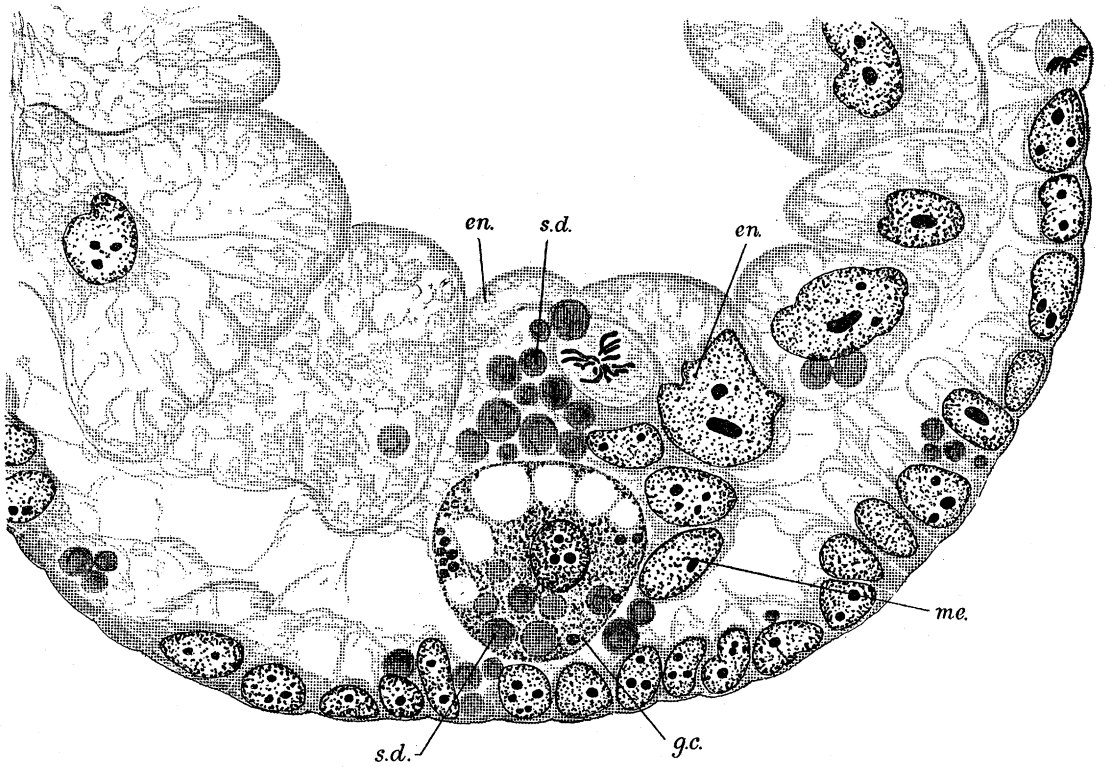

FIGURE 3. Transverse section of *P. capensis* at the same stage as that shown in figure 123, plate 41 at the level indicated (*t.f.*3). The section passes just behind the blastoporal area from which immigration is taking place, and through the 'giant cell' (*g.c.*) which lies close to the ectoderm (cf. figure 123); secretory droplets of various sizes lie in the cytoplasm of this cell and outside it, and vacuoles indicate their site of formation. Four mesodermal nuclei are shown, one in mitosis, and three nuclei of endodermal cells lie outside the plane of the section. The corresponding stage for *P. balfouri* is shown in figures 107 and 108, plate 40. × 320 approx.

lip has given rise to about twenty-six mesodermal cells, four of which are seen in the section shown in figure 123, plate 41, and to a 'giant cell'. This stage corresponds to that of the dilated embryo of *P. balfouri* (*m.*) on figures 76, and 107 and 108, plates 37 and 40. Immigration from the blastoporal area of *P. capensis* takes place mostly in the middle line, and so a 'primitive groove' appears as Sedgwick described, which differs from the blastoporal area of the other species where immigration occurs more evenly over a wider area and no marked furrow appears.

The giant cell (*g.c.*) in *P. capensis* may lie closer to the endoderm (figure 123, plate 41) or to the ectoderm (figure 3), and occupies the posterior part of a small group of mesodermal cells; it is probably one of the first cells to become internal from the blastoporal area, as in *P. balfouri*. It is clearly an active gland cell. It is about 30μ in diameter with a pale staining

unlobed nucleus, and the cytoplasm, unlike that in *P. balfouri*, is densely packed with mitochondria. Numerous secretory droplets (*s.d.*) of various sizes are present in the cytoplasm, and many similar droplets lie outside the cell; they stain brown with haematoxylin. The transverse section in figure 3 shows a giant cell which appears to have just discharged many droplets, as a number of these lie near it leaving empty vacuoles in the marginal parts of the giant cell. These droplets are abundant near the giant cell, and spread round the sides of the mouth-anus and elsewhere, and can be found incorporated into the cytoplasm of both ectodermal and endodermal cells. No such droplets are present before the giant cell appears. Embryos showing the giant cell are 286 to 396μ in length; this stage was not seen by Sedgwick. The giant cell disappears completely as in *P. balfouri*, and no trace is present in embryos 424μ in length; its secretory products are also soon absorbed. No suggestions can be offered as to its function.

After the disappearance of the giant cell the 'primitive groove' elongates and becomes a little removed from the mouth-anus as in the other species (plates 32, 34 and 38). Mesodermal immigration continues, forming a circular patch of undifferentiated cells behind the mouth anus. Such a stage is intermediate between those reconstructed in figures 113 and 114, plate 41. When slightly older, some germ cells appear in this circular patch of mesoderm, but they are scattered and do not form a compact rudiment as in the other species, and the limits of the genital rudiment are less sharp than the reconstructions on plate 41 may suggest. The nuclei of the germ cells are pale staining as in the other species, and their cytoplasm is rather dense and often stains more brown than grey with iron haematoxylin. Mesodermal bands are formed as described on p. 519, and the growth of the germinal bands resembles that of *P. balfouri* (p. 508).

A close series of embryos has been examined to ascertain whether any endoderm is formed by immigration from the blastoporal area (primitive groove). As in the condensed embryo of *P. balfouri* (see p. 508), no indication of such a process could be found. It is possible that a few endodermal cells may arise in this way, but too slowly to be detected by the available methods. It is clear that the bulk if not the whole of the definitive endoderm arises from the early vacuolated cells formed during segmentation which take up their position in association with a blastopore. A consideration of the different modes of formation of endoderm in the species of *Peripatopsis* is given on pp. 513, 536 and 539.

Formation of the mouth-anus opening (blastopore of earlier workers) and its development into mouth and anus

The mouth-anus opening, assumed to be the blastopore by Balfour (1883) and Sedgwick (1885) (see pp. 530 and 532), has not previously been traced to its origin. In *P. capensis* a conspicuous blastopore closes as shown on p. 503. Possibly it reopens to form the mouth-anus later, or the latter may be formed on its site. In *P. balfouri* a homologue of the blastopore is early obliterated, and in *P. moseleyi* and *P. sedgwicki* this feature is not developed. The origin of the mouth-anus in these three species, which all possess a dilated blastodermic vesicle, has been traced in detail. It arises *de novo*, after endoderm formation has considerably advanced, and it is formed in essentially the same manner in all three species, although transitory vacuolated cells are formed in *P. balfouri* but not in *P. sedgwicki* or *P. moseleyi*.

Later stages only have been seen of the species shown in figure 5*a* to *d*, and there is no reason to suppose that the origin of this organ is not substantially the same as in the other species with a large blastodermic vesicle.

The position of the mouth-anus has been described above (p. 504). The sheet of endoderm spreading from the blastoporal area comes to lie below the anterior thickening of the germinal disk. Here a small area of endoderm thickens by the closer approximation of its cells, and is situated against a similar thickening of the ectoderm. In *P. moseleyi* and *P. sedgwicki* this ectodermal thickening is at first relatively slight, but in *P. balfouri* it forms the greater part of the anterior thickening of the germinal disk. These features are shown in the transverse section of *P. sedgwicki* in figure 55, plate 36, at the level indicated in figure 21, plate 32, the middle line being marked by the arrow *m*. The endoderm is clearly thicker near the middle than laterally, and there is no trace of a connexion between the endoderm and the overlying ectoderm. The endodermal sheet at this stage has reached the area surrounded by the dotted line in the reconstructions of *P. balfouri* (figures 74 and 75, plate 37), but lies far beyond the limits of the reconstructions shown in figure 40, plate 34 onwards for *P. moseleyi* and figure 21, plate 32 onwards for *P. sedgwicki*, where it lines up to half of the blastodermic vesicle.

As the ectoderm and endoderm thicken, some cells become orientated about the middle line, starting anteriorly. No nuclei lie on a short sagittal line, which marks the future mouth-anus, and cells take up positions on either side of it radiating outwards anteriorly and laterally. An early stage of this orientation is seen in the ectodermal cells of a whole mount shown in figure 4 (only a small part is drawn; for its position see the dotted rectangle *t.f.*2 in figure 22, plate 32). Anteriorly a row of cells form a clear boundary to the mouth-anus, which is starting to perforate, and posteriorly the lips are not yet defined, but a mitosis lies on the right side where the lips will be formed. A thickened layer of endodermal cells becomes similarly orientated, thus leaving only apposed cell boundaries along a sagittal line. These orientated cells are indicated by broken radiating lines on the reconstructions, figures 21 to 23 and 40 to 45, plates 32 and 34.

On either side of this sagittal line the ectodermal cells tip inwards and the endodermal cells outwards so that the two layers unite to form the lips of the mouth-anus, as shown at *l.* in the transverse section in figure 56, plate 36 of *P. sedgwicki* cut in the plane indicated by the arrow in figure 22, plate 32. Where the ectoderm and endoderm are united to form the lips, a furrow lies externally (figures 57, plate 36 of *P. moseleyi*). The extent of the formed lips is shown by a heavy sagittal line in the reconstructions (figures 22 and 40 to 45, plates 32 and 34). On either side and in front and behind the lips the ectodermal and endodermal layers are in contact but not united (see the lateral regions of the transverse sections in figures 56 and 57, plate 36 and the anterior region of the sagittal section, figure 54, plate 35).

The lips vary in depth, they comprise about one row of ectodermal and one of endodermal cells in *P. balfouri* (figure 109, plate 40), there are about two rows of cells in each layer in *P. sedgwicki* (figure 56, plate 36), and in *P. moseleyi* the lips deepen and comprise many rows of cells as seen in figure 57, plate 36. The right and left lip edges separate early in *P. sedgwicki*, *P. balfouri*, *P. capensis* and in the species shown in figure 5*a* to *d*, but they remain apposed in *P. moseleyi* until after the division to form the mouth and anus. In the two former species the lips separate first in the anterior part of the mouth-anus, forming a small longitudinal slit which opens into the fluid-filled yolk sac. This opening is at first traversed by strands

of protoplasm. In *P. sedgwicki* these are scanty and soon disappear. In *P. balfouri* the lips draw apart almost as soon as they are formed, but they are united by a continuous delicate sheet of protoplasm (figure 109 *c.m.*, plate 40 and figures 76 and 78, plates 37 and 38); the whole zone appears to be under considerable tangential tension, probably due to the swelling of the blastodermic vesicle. When the latter suddenly shrinks (see p. 512) the lips come together as in figure 77, plate 37, and later diverge again with further growth in figure 78, plate 38. The uniting membrane disappears at stage *p* and the mouth-anus freely

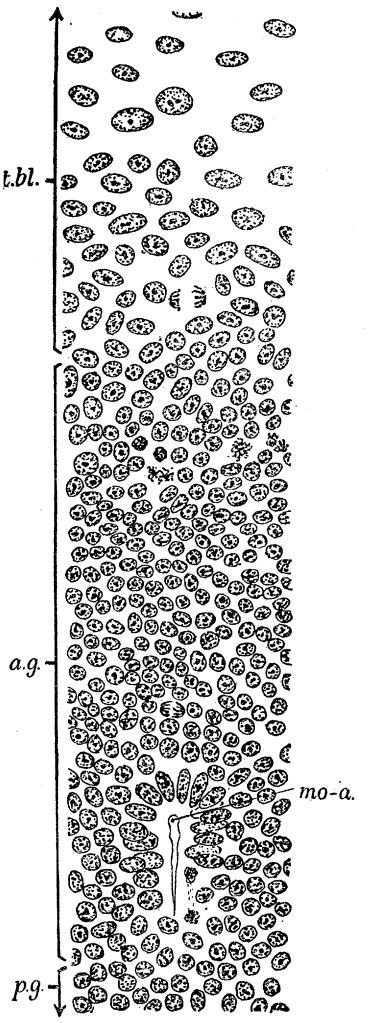

FIGURE 4. Surface view of part of a whole mount of the germinal disk of *P. sedgwicki*, a little younger than that shown in figure 22, plate 32 on which the corresponding area is enclosed by a dotted rectangle (*t.f.*4). The superficial nuclei only are shown, those lying on the thin blastoderm (*t.bl.*) which extends outside the germinal disk, appear large because they are tangentially flattened, and the crowded nuclei of the germinal disk (*a.g.* and *p.g.*) are radially elongated and thus appear small. The mouth-anus (*mo.a.*) and its lips are becoming organized from before backwards. The blastoporal area lies posterior to the zone figured (see figure 22, plate 32).

communicates with the interior. In both species the mouth-anus is widest at the anterior end. It gradually elongates and reaches maximum dimensions at a little beyond stage *E'* with one somite for *P. sedgwicki*, at stage *q* for *P. balfouri*, and at the stage shown in figure 116, plate 41 for *P. capensis*. In *P. moseleyi* the apposed lips reach a maximum length a little beyond stage *L* with five somites.

The size of the mouth-anus differs widely in the several species as shown in the plates. That of *P. capensis* becomes enormous and widely open (figures 114 to 117, plate 41); the lips here protrude and curl slightly outwards, as seen in the transverse section (figure 119, plate 41). In the species shown in figure 5 *a* to *d* the mouth-anus resembles that of *P. moseleyi*

in general form, but it is open instead of closed, and internally the endoderm is thrown into folds just within the lips (*e.f.*, figure 5*d*, p. 524).

The mouth-anus rudiment divides into mouth and anus by the fusion together of the greater part of the lips in the middle line, as figured by Balfour (1883) for *P. capensis*, and shown here in figure 118, plate 41 for *P. capensis*, and figure 5*b* for the undescribed species, leaving a conspicuous open mouth and anus, seen also in *P. balfouri* (plate 38). In *P. sedgwicki* the mouth is large and elongated, but the anus is minute and forms a narrow oblique tube with the internal opening anterior to the external one as shown in figure 25, plate 32, both mouth and anus are formed by the time the third pair of somites are differentiated (figure 24, plate 32). In *P. balfouri* the mouth and anus are formed just before the first pair of somites are differentiated, and the mouth, which is larger than the anus, is rounded from the start (figure 83, plate 38). In *P. moseleyi* the mouth and anus separate between stages *L* and *M* when 10 to 15 pairs of somites are present, the lips remaining apposed. The anus rudiment is larger than that of the mouth at stage *M* with sixteen somites, and when nineteen pairs are formed the lips of both mouth and anus part leaving a small circular mouth and elongated but smaller anus (figure 46, plate 34). Where the lips of the mouth-anus fuse across the middle line, the ectoderm and endoderm of either side unite once more; this region then becomes thinner (figure 112 *v.b.*, plate 10) and indistinguishable from the ventral walls of the yolk sac between the arms of the germinal band.

The line of union of the ectoderm and endoderm at the edges of the mouth-anus may be clearly seen histologically at many stages of development, although not at first detectable in the mouth-anus rudiment of *P. sedgwicki* and *P. moseleyi* (figures 56 and 57, plate 36). In *P. balfouri* the longitudinal sections (figures 110 and 111, plate 40) show a junction of the layers at the margin of the mouth-anus, and in *P. capensis* the junction is equally clear but is reflected outwards by the protrusion of the lips (figure 119, plate 41). In *P. balfouri*, when the mouth-anus has divided, the junction of the layers is seen with diagrammatic clarity round the anus and at the posterior margin of the mouth (figure 112, plate 40), but anteriorly the ectoderm has tucked in to form the stomodoeal rudiment (as noted by Sedgwick), and the union with the endoderm is here well within the mouth opening. The stomodoeal intucking increases in extent with advancing age. Similar conditions are found in the other species. The further development of the endoderm, stomodoeum, and proctodoeum have been described by Sedgwick (1887).

Evidence against the lips of the mouth-anus giving rise to endoderm

The assumption by earlier workers that the mouth-anus is a blastopore is coupled with the view that the lips of this organ supply much of the endodermal layer. Mitoses are frequent in the lips of the mouth-anus (figures 54, 56 and 57, plates 35 and 36), but they occur in both ectoderm and endoderm, and there is no indication that endoderm is thereby invaginating. In *P. sedgwicki* and *P. moseleyi* the mouth-anus starts to develop just before the endodermal layer has spread throughout the blastodermic vesicle, but the vesicle is completely lined by the time the mouth-anus is fully established in *P. sedgwicki* (figure 23, plate 32), and the mouth-anus is still rudimentary in *P. moseleyi* when the endoderm is at this stage (figures 31 and 32, plate 33). Thus in these two species the mouth-anus arises too

late to have any connexion with the formation of the endodermal lining of the blastodermic vesicle. In *P. balfouri* the mouth-anus is formed when the endoderm is less extensive, but, as in the other species, this layer is present below the anterior thickening of the germinal disk *before* the mouth-anus develops, and is not formed as a result of mouth-anus development. In *P. capensis* it has been shown (p. 503) that the endoderm is completed by the time of closure of the blastopore, so that in this species also the mouth-anus is formed *after* the definitive endoderm is established.

The abundance of mitoses in the lips of the mouth-anus is correlated with the great growth of the lips, first to form the relatively large mouth-anus, and secondly, when the mouth has separated from the anus, the middle region of the lips gives rise to the whole of the ventral surface of the body between these openings. There is thus clear evidence of the origin of the whole of the endodermal layer from the blastoporal area or primitive streak by immigration in the three species with large blastodermic vesicles, and no evidence of any invagination from the lips of the mouth-anus, even in *P. capensis*.

A confirmation of this conclusion is provided by two abnormal embryos of *P. balfouri* in which no mouth-anus or anterior thickening of the germinal disk are present. In one at stage *m* the posterior thickening is normal in every way showing typical development of endoderm and mesoderm. In the other at stage *o* the condensed embryo is fully lined by endoderm and the blastoporal area and mesoderm are also normal, but there is no trace of the mouth-anus which should be large and open at this stage. In an older *P. moseleyi* (figure 5*e*) four or five pairs of abnormal mesodermal somites (see p. 525) are present, also typical genital rudiment and endoderm, but there is no mouth-anus. Where this organ should be there is no break in the ectoderm and no lip organization, although a trace of an ill-formed and irregular thickening of both layers can be detected at *r*. If the mouth-anus was concerned with endoderm formation the latter process would not be expected to proceed normally in its absence. The evidence against the mouth-anus playing any part in the formation of endoderm thus appears conclusive.

Formation of mesodermal bands and germ cells

Some general considerations concerning the mesoderm formation and the earliest stages of immigration from the blastoporal area have already been made (p. 509). The first few cells to become internal give rise to endoderm in species other than *P. capensis*, and are seen in longitudinal section in figure 48, plate 35 for *P. moseleyi* (*P. sedgwicki* is practically identical), and in the transverse sections in figures 106 and 107, plate 40 for *P. balfouri*. In *P. sedgwicki* the formation of mesoderm is most simply seen, while the early appearance of the 'giant cell' in *P. balfouri* and *P. capensis* and of the genital rudiment in *P. moseleyi* cause differences in the general appearance. Mesodermal immigration from the blastoporal area is shown by horizontal lines in the reconstructions and the genital rudiment by heavy dots.

Mesodermal cells immigrate from the posterior and middle parts of the blastoporal area behind the endodermal immigration (figures 54 and 59, plates 35 and 36), or from the whole of the blastoporal area in *P. capensis*, but in early stages the immigration of endodermal and mesodermal cells cannot be differentiated. This process leads to the formation of a small, roughly circular, mass of mesodermal cells lying internal to the whole or only to the

posterior part of the blastoporal area and behind it, seen in figures 21 and 54, plates 32 and 35 for *P. sedgwicki*, figure 42, plate 34 for *P. moseleyi*, and figures 113 and 123, plate 41 for *P. capensis*. In *P. balfouri* it at first lies internal to most of the blastoporal area (figures 75 and 79, plates 37 and 38). The cells are even in size with darkly staining cytoplasm, and clearly different in appearance from both the ectodermal and endodermal cells (see figures 54 and 57 to 59, plates 35 and 36). For *P. sedgwicki* a stage a trifle older than that of the reconstruction in figure 21, plate 32 is shown in longitudinal section in figure 54, plate 35; the mesoderm (*me.*) lies between the superficial blastoderm and the endoderm, and is confluent anteriorly with the immigrating cells of the blastoporal area (*b.a.*). This mass of mesodermal cells increases by mitoses and further immigration, as seen in the transverse section of *P. moseleyi* (figure 59, plate 36), and spreads farther outwards, mainly posteriorly and laterally (figure 22, plate 32). A lateral expansion then predominates, followed by a forward growth on either side to give the mesodermal bands at the sides of the mouth-anus (figures 23, 43 and 59, plates 32, 34 and 36). The blastoderm overlying the mesodermal bands becomes thicker than elsewhere, forming with the mesoderm the germinal bands. The shape and movements of the latter during early stages of development have already been described (p. 506).

The germinal disk, and the whole embryo in *P. capensis*, is first of even thickness as described above (p. 513). As the mesodermal bands develop the overlying blastoderm becomes correspondingly changed, forming the thick germinal band ectoderm and thin walls of the yolk sac or of the remains of this organ in *P. balfouri* and *P. capensis*. The organization of mesoderm and ectoderm takes place simultaneously, but there is every indication that it is determined by the mesoderm. No ectodermal differentiation of the germinal band from the undifferentiated germinal disk takes place prior to the arrival of the mesoderm. If this is so, then there is no support from *Peripatopsis* for Snodgrass's contention that metameric segmentation arises in the ectoderm (see discussion, p. 552) and is secondarily impressed on the mesoderm.

The genital rudiment is differentiated early in *P. sedgwicki*. It arises from some of the mesodermal cells situated posteriorly in the mesodermal mass, shown by the dotted area in the reconstruction in figure 22, plate 32, and seen in longitudinal section in figure 54, plate 5 of a slightly older stage (*g.*). The germ cells are larger than the undifferentiated mesodermal cells and usually stain much paler, the large lightly staining nucleus being particularly characteristic, as shown by the sections in figures 54, 59 and 112, plates 35, 36 and 40. Germ cells are not always visible in early stages as clearly as shown in these figures, but in later stages they usually appear as described. The genital rudiment increases in size by mitosis and probably also by differentiation of further mesodermal cells.

In *P. balfouri* the establishment of a U-shaped mesodermal band occurs as in *P. sedgwicki*. Mesoderm formation starts on the fully dilated blastodermic vesicle (stage *m*, plates 37 and 40), and when this has condensed to form the small embryo (stage *o*), a group of mesodermal cells lies behind the open mouth-anus (figures 79 and 110, plates 38 and 40). In the section all tissues are seen to be much thinner than in the corresponding longitudinal section of *P. sedgwicki* (figure 54, plate 35), which otherwise differs only in that the lips of the mouth-anus are not yet fully organized and that the genital rudiment is differentiating. In *P. balfouri* the genital rudiment arises as in *P. sedgwicki*, but later, when eight pairs of somites are formed (*g.*, figures 91 and 112, plates 38 and 40). The germ cells first appear on the inner

side of the mesodermal mass internal to the blastoporal area and approximately coextensive with it.

In *P. capensis* mesodermal bands are formed as in *P. balfouri*, but the genital rudiment arises earlier, being formed before the cavities of the first somites appear (see also p. 515).

In *P. moseleyi* the genital rudiment is differentiated before the main mass of undifferentiated mesoderm has appeared. The first cells to pass in posterior to the endodermal immigration at once form a spherical group of germ cells, with the cytological characteristics noted above, and lie just posterior to the short blastoporal area (figure 39, plate 34). A section of this stage is shown in figure 49, plate 35, where the genital rudiment comprises seventeen cells and twenty-three endodermal cells are present. An older stage is shown in longitudinal section in figure 50, where the endoderm has spread far beyond the figure and the genital rudiment is larger; the blastoporal area is larger, but it is still short, and the cells streaming in supply endoderm and possibly further germ cells, but a mitotic figure indicates multiplication within the genital rudiment itself. The lips of the mouth-anus are not yet formed. After this stage no further germ cells have been seen to arise by immigration. Mesodermal cells now pass in from the blastoporal area and form a disk between the genital rudiment and the blastoderm, just as in the other species, shown in figure 40, plate 34. (The corresponding stages for *P. sedgwicki* are seen in figures 21, 22 and 54, plates 32 and 35, and for *P. balfouri* in figures 75, 79 and 110, plates 37, 38 and 40.) This layer of mesoderm is irregular and several cells thick, and presents the same cytological features described above (p. 520). The mesoderm extends first in all directions behind the mouth-anus (figure 42, plate 34), then mainly laterally, and later forwards to form the mesodermal bands as in the other two species. The genital rudiment grows in an irregular manner forming a roughly median mass (figures 43 and 44, plate 34). A transverse section of stage *K* (figure 59, plate 36) at the level indicated on figure 43, plate 34 shows the genital rudiment cut twice, the mesoderm has spread laterally and merges with the immigrating cells of the blastoporal area, and mitoses are present in the genital rudiment, mesoderm and ectoderm. The edge of the germinal disk lies at the side of the figure where the ectoderm is becoming thinner.

Formation of mesodermal somites

The formation of the mesodermal somites has been studied in detail in a series of embryos of the five species of *Peripatopsis*, but only a few reconstructions can be reproduced here. This detail is needed (1) to ascertain whether 'primary' and 'secondary' segmentation occurs in the Onychophora (see p. 546), and (2) for a fuller consideration of the composition of the head in the Onychophora and other Arthropoda. The formation of somites from the mesodermal bands occurs in the same general way in all five species. Considerable individual and bilateral differences occur, particularly in the earlier stages, which are standardized later. Each species has its own pattern of development and range of variations; the size of the embryos influences the number of cells at any one level of a mesodermal band, and the number of cells present influences the origin of a coelomic cavity from one or from several initial spaces.

When the U-shaped mesodermal bands reach the level of the anterior half of the mouth-anus, or the equivalent position between the separated mouth and anus, the cavities of the

first pair of somites begin to form. One initial space appears in the small embryos of *P. balfouri* (figure 84, plate 38), where few cells are present, and several may appear in the larger species with more cells (see figure 116, plate 41 for *P. capensis* and figure 43, plate 34 for *P. moseleyi* and figure 5*b*). The several spaces become confluent as the bands grow forwards (figures 44, 45 and 117, plates 34 and 41). The mesoderm around these cavities either becomes completely nipped off from the band behind (*P. balfouri*, figure 86, plate 38 and *P. moseleyi*, figure 43, plate 34) or remains demarcated from it by a narrow neck which is later severed, as in *P. capensis* (figures 117 and 118, plate 41).

As the bands grow forwards the cavities of the succeeding somites appear. They are usually smaller than those of the first pair, and the anterior few pairs tend to arise in quick succession, or almost simultaneously (figures 5*b* and 5*c*, 25, 114 to 116, plates 32 and 41), but do not contrast in any way with those of the more posterior somites.

The simplest course of events is shown by *P. sedgwicki* (figures 24 to 26, plate 32), where the paired somites arise very evenly, one by one, from before backwards, and each starts with one initial space. The mesodermal bands on either side become progressively shorter at later stages, so that by stage *H'* onwards a somite is cut off as soon as sufficient mesoderm has migrated to a lateral position. The even development of somites 5, 6 and 7 is shown particularly clearly. This even and progressive formation of the anterior somites is also shown in the species illustrated in figure 5*a* to *d* despite the irregularities. (This species shows greater individual variations than do the others.) The germinal disks here are a little larger than in *P. sedgwicki*, but the general features are the same.

In *P. moseleyi*, *P. balfouri* and *P. capensis* somites 2 to 5 tend to arise nearly simultaneously, but many embryos of *P. moseleyi* show rudiments of the cavities of somite 2 (figure 43, plate 34) or of somites 2 and 3 before those of somites 4 or 5. The cavities of somites 2 to 5 appear in each lateral band before it shows clear indications of dividing into separate blocks (see *P. capensis*, figures 115 to 118, plate 41). There may be one rudimentary cavity to each somite (figures 86, 87, 89, 91 and 115, plates 38 and 41) or there may be more (figure 116, plate 41); *P. moseleyi* also provides examples of both alternatives. Two embryos of *P. balfouri* figured show variations in detail, one has four separated hollow pairs of somites and a fifth clearly indicated but still united with the band (figure 86, plate 38; the fifth somite is not clearly seen at this angle), and the other has five pairs of hollow somites but the second to fifth are not yet separated from each other (figure 87, plate 38).

In *P. capensis* and *P. balfouri* stages occur with five pairs of somites and a mesodermal band conspicuously elongated transversely (figures 87, 116 and 118, plates 38 and 41). It is not considered that these appearances indicates any distinction between the first five somites and the rest. It is a difference of degree and not of kind. The mesodermal bands become progressively shorter in all species as more somites are formed. When the first four or five somites arise almost simultaneously, a close series of stages shows that they rarely if ever appear quite simultaneously, and *P. sedgwicki* and the species shown in figure 5*a* to *d* clearly show no distinction between the time or the manner of origin of the first few somites.

The multiple origin of the coelomic spaces of the anterior somites of *P. moseleyi*, *P. capensis*, etc., is no evidence that such a somite represents the mesodermal components of more than one segment. The initial cavities are variable in numbers and in position, and the somite once established with its large single cavity is clearly homologous with the somites in similar

positions in the species where the initial cavity is single. This fact is of theoretical importance (see discussion p. 556).

The cutting off of each somite from the mesodermal band usually occurs relatively earlier in the more posterior somites in all species, so that separate solid blocks appear first and their cavities develop immediately after (figures 26, 45 and 46, plates 32 and 34). However, exceptions occur, and one embryo of *P. moseleyi* at the stage shown in figure 45 has been found with only traces of coelomic spaces in the posterior seven or eight pairs of somites, while the anterior seven pairs show large cavities. The condition here of the posterior somites resembles the more frequent almost simultaneous development of cavities in the anterior four or five pairs of somites. As the cavities of the somites increase in size their walls become one cell thick, as in figure 58, plate 36. Against the endoderm they become thinner, but remain thick against the ectoderm as shown by Sedgwick.

The paired somites shift forwards in a similar manner in all species. The first pair approach each other anterior to the mouth and establish the antennal segment (figures 44 to 46, 86, 89 and 91, plates 34 and 38). The second pair are smaller and lie at the side of the mouth where they establish the mandibular segment. The third pair are larger than the second and all succeeding somites in early stages in some species (figures 44 to 46, plate 34), and establish the slime papilla segment. The positions of the somites and germinal bands are shown in the reconstructions, and their changing positions in three species have been outlined above (p. 506) in the account of the germinal bands. The undifferentiated bases of the germinal bands close to the blastoporal area become shorter and together form a roughly transverse mass of tissue in *P. moseleyi* and *P. sedgwicki*, and a U-shaped mass in *P. balfouri* and *P. capensis* where so little yolk sac is present. The cavities of the last pair of somites appear in the base of the mesodermal bands and immigration from the blastoporal area then ceases (figure 47, plate 34). The mesoderm just within the disappearing blastoporal area becomes incorporated into the last pair of somites. It is possible that a few cells supply a minute posterior unsegmented region (corresponding with a telson) behind the last segment, but the limits of such a region have not been defined in the early stages here described (see p. 555). Clearly the initiation of metamerism lies in the mesoderm, as no ectodermal segmentation is established until after the mesodermal somites have completed their migrations (see p. 552).

Considerably more minor asymmetry can be found at the anterior end of the body than in the middle or posterior parts. Striking asymmetry of the anterior somites occurs in early stages, particularly in the species shown in figures 5 *a* to *d*, and in *P. moseleyi* where the axis and position of the germinal disk upon the blastodermic vesicle is so varied. The first few pairs of somites may differ greatly in size on the two sides, and may be situated at different levels. These asymmetries are later rectified as the yolk sac becomes absorbed. Other asymmetries are definitely abnormal, and probably lead to death of the embryos. A *P. sedgwicki* has been found with four typical somites on one side and a short solid unsegmented mesodermal band on the other. A *P. balfouri*, although externally symmetrical, has four somites on one side and on the other a large single somite. A *P. moseleyi* with a long mouth and possibly a minute anus has one pair of fairly symmetrical somites occupying the normal space of five or six pairs; between their posterior ends lies an irregular blastoporal area, and another small pair of somites together with a median large somite lies

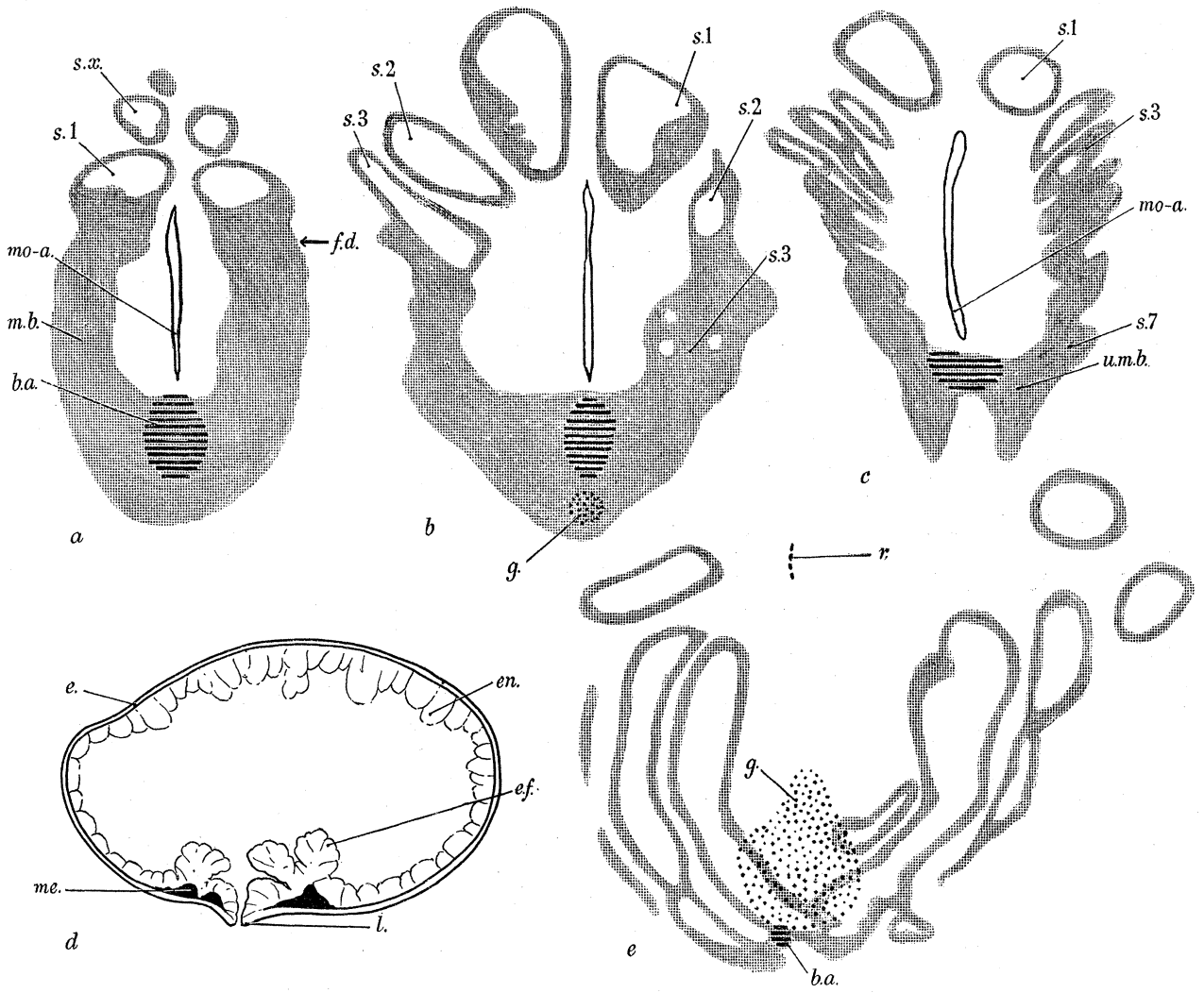

FIGURE 5. *a* to *c* represent reconstructions of the germinal disks of an undescribed species of *Peripatopsis* with sixteen legs occurring near Grahamstown (see p. 487 and Manton 1938*b*). Horizonal hatching represents mesodermal immigration and mesoderm is shaded. × 105 approx. *a*. The open mouth-anus is about to divide by fusion of the middle part of the lips. The mesodermal bands show anteriorly the cavities (*s.* 1) of what appear to be the antennal somites by their size and position, just in front of the mouth-anus (cf. reconstructions of the other embryos and species). In front of these lie a pair of small hollow somites (*s.x.*) and an asymmetrical solid mass of mesoderm, all of which are considered to be abnormalities (see text). It is improbable that the somites (*s.x.*) represent the antennal somites as they are too small and lie too far forward, if they are so interpreted, then the somites marked *s.* 1 are too pre-oral to be regarded as the jaw somites. No genital rudiment could be detected but the fixation is not perfect. *b*. A slightly older embryo. Three pairs of somites are either separated or indicated by coelomic cavities, and the third on the right is starting from three initial spaces. A genital rudiment is present. *c*. Older embryo showing fairly even development from before backwards in spite of the irregularities, seven pairs of somites are formed or indicated. A genital rudiment could not be detected. *d*. Transverse section of the embryo shown in *a* at the level indicated. The endoderm (*en.*) is thrown into folds (*e.f.*) within the lips (*l.*) of the mouth-anus, the mesoderm is black and the ectoderm white. *e*. Reconstruction of an abnormal embryo of *P. moseleyi*. The endodermal layer is normal. Mesoderm is still immigrating from the blastoporal area; the mesoderm already formed is entirely abnormal, hollow somite-like sections lying longitudinally through the disk. A large genital rudiment is present. No mouth-anus is formed, but an abnormal trace of a mouth-anus rudiment is found at *r.* in which there is a small irregular endodermal and ectodermal thickening, but no union of the two layers. For normal embryos of the same stage (see plate 34).

behind this zone, the germ cells being normal. Another *P. moseleyi*, referred to on p. 519 (figure 5*e*), lacks a mouth-anus and shows four or five pairs of somites, two or three of which are elongated antero-posteriorly. Such embryos probably could not rectify to give normal individuals.

No trace of mesoderm lies anterior to the first or antennal pair of somites in any embryo examined of *P. sedgwicki*, *P. moseleyi*, *P. balfouri* or *P. capensis*. A single embryo of the undescribed species shown in figure 5*a* does show this feature. Coelomic cavities, whose size and position suggest that they will form the antennal somites, lie at the anterior end of mesodermal bands, but in front of them lie a small pair of hollow somites, and on one side only a smaller block of solid mesoderm. No such structures are present in older embryos. In view of the extensive irregularities occurring occasionally in all species and in an exaggerated form in this species, no significance can be attached to this isolated case of pre-antennal mesoderm.

The genital rudiment

The origin of the germ cells from the undifferentiated mesodermal cells at the base of the U-shaped mesodermal band has been described in *P. sedgwicki*, *P. balfouri* and *P. capensis* (p. 519 and figures 54, 112 and 114, plates 35, 40 and 41). At first they form part of the mesodermal mass, lying against the endoderm. In *P. moseleyi* the germ cells arise before the mesoderm by immigration from the blastoporal area (p. 521), but later the genital rudiment lies in the same position as in the other species (figures 23 to 26, 39 to 43, 91, 114 to 117, plates 32, 34, 38 and 41 and figure 5*b**). Once the rudiment is established it grows by division of its cells, giving much more tissue than is later utilized in formation of the gonads. The further growth of the genital rudiment has been followed in some detail in *P. moseleyi* only, but its development in the other species probably does not differ materially. The genital rudiment when first established comprises fewer cells in the smaller species, and in later stages correspondingly fewer germ cells pass into the somite walls (see below).

The genital rudiment in *P. moseleyi* tends to shift postero-dorsally, either as a continuous mass or by fragmentation into smaller portions containing from one to many cells. A few germ cells may remain near the blastoporal area, but the majority lie between the ectoderm and endoderm of the dorsal yolk-sac wall, as seen in figure 45, plate 34. A large mass may be present, as in this embryo, which persists for a long time lying just behind the forward flexure of the body. Or, alternatively, small groups of germ cells may be either scattered in between the dorsal extremities of the somites in the hinder region of the body, as in figure 46, plate 34, lying between these somites and the endoderm (appearing superimposed on the somites in the figure), or they may all pass farther away from the blastoporal area, reaching the wall of the yolk sac postero-dorsal to the ninth somites, and beyond the point marked *x* in figure 46.

Some of the free single germ cells come in contact with the mesodermal somites, either at their dorsal extremities or by passing between the endodermal layer and the somite walls. Here they at first lie in close contact with the mesoderm, as seen in somite 17 on figure 58,

* The genital rudiment in figures 115 to 117 (heavy dots as in figure 114) scarcely shows in the reproductions of these figures.

plate 36, and then they become incorporated into the wall, as seen in somite 18. Germ cells which have become part of the somite wall are shown by black rectangles in the reconstructions in figures 46 and 47, plate 34. They are distributed with great irregularity. Table 1 shows the numbers of germ cells in the somite walls in a series of embryos of advancing ages. As more germ cells become incorporated into the somites, fewer remain free, but no free germ cells occur in embryo 26·1 which has only twenty-two germ cells in its somite walls, while twelve remain free in embryo 22·1 which has seventy-eight germ cells in its somite walls. The free germ cells become fewer and finally disappear. As in many other arthropods, only a small proportion of the germ cells present in the early connected rudiment become

TABLE 1

This table shows the distribution of germ cells, both free and in the somite walls, of a series of embryos of *P. moseleyi*. The vertical lines mark the posterior end of the body at each stage. The number of segments in the adult *P. moseleyi* varies between 23 and 27 (20 to 24 bearing legs behind the oral papillae). Embryos 18·2 onwards have completed the posterior end of the body. The two columns of figures under each segment represent the numbers of germ cells in each of the pair of mesodermal somites of that segment.

reference numbers of embryos	figure on the plates	hinder body segments 10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	total germ cells in somite walls	total germ cells free	
19·2	43	—	—	1.	—	—	—	1.1	—	—	—									3	many	mesodermal
19·3		—	—	—	—	—	—	—	—	—	—	—								0	many	immigration
10·2		—	—	—	—	—	—	—	—	—	—	—	—							0	few	in progress
9·1		—	—	—	—	—	—	—	1.1	—	—	1.1	1.1	—						6	many	from the
22·2		—	—	—	—	—	—	—	—	—	—	—	1.	—	—					1	many	blastoporal
18·1		—	—	—	—	—	—	—	2.	2.2	.3	4.2	—	.7	11.6	11.8	8.7			75	8	area
18·2		—	—	—	—	—	—	—	2.1	2.	1.	—	1.	1.	7.6	7.10	3.2	5.6		54	36	immigration
19·1	44	—	—	—	—	—	—	2.	—	—	—	—	—	2.1	1.2	8.2	2.5	3.2		30	20	ceased, all
16·1		—	—	—	—	—	—	—	.1	—	3.2	1.3	.3	.3	2.9	3.6	.11	9.4		60	35	somites
26·1		2.2	1.	.2	1.2	1.2	.1	.1	.2	.1	—	—	—	1.	2.	1.				22	0	formed
22·1		—	—	—	—	—	—	—	1.1	—	1.1	2.2	2.4	4.6	10.8	9.4	7.5	7.4		78	12	

incorporated into somites. The somites which take up the germ cells lie behind the ninth pair, but most germ cells come to lie in those behind the fifteenth pair. The embryo of *P. balfouri*, shown in figure 96, plate 38, corresponds in age to that of *P. moseleyi* 18·2 in the table, but it possesses only twelve germ cells in the somite walls; they lie in segments 15 to 20 with a maximum of three germ cells in one somite and there are no free germ cells.

ABNORMAL EMBRYOS

Considerable variations occur in all stages of development which appear to be regularized with further growth to give normal young. Some of these individual and bilateral variations which appear to be slowly eliminated have been recorded above (pp. 507, 521 and 522). Other variations are clearly gross abnormalities, and could not give rise to normal young (pp. 519 and 523). These variations and abnormalities are not due to the effects of years of life in a terrarium, as they are frequent in animals freshly collected from the wild. They are described here because they provide information concerning theoretical matters (see pp. 519 and 555). Some abnormal unsegmented ova are recorded on p. 497.

Discussion part 1, The Onychophora

Membranes of the egg and embryo

It would be profitless to review the numerous descriptions of onychophoran embryonic membranes without further information concerning the properties of these membranes, their places of origin, and their duration, in forms other than *Peripatopsis*. Dendy (1902) compared the membranes of the Australian Onychophora, and Bouvier (1904) reviewed at some length the available information and found it impossible to reconcile the various accounts. He pointed out that the term 'chorion' had often been badly applied, and noted the desirability of conforming with accepted terminology, quoting Korschelt and Heider's definitions of 'vitelline membrane' as a structure arising from the peripheral protoplasm of the egg, the 'chorion' as arising from a 'follicular secretion', and a tertiary membrane of 'shell' formed within the ovarian or oviducal cavity. It is, however, impossible to apply this terminology to the membranes of the Onychophora, and a final naming of the various membranes here has as far as possible been avoided.

There appear to be as many as four different membranes round the ova and embryos of various Onychophora, and not two as previously supposed, and their relative thicknesses in the known species are very different:

(1) The membrane formed round the ova in the ovarian follicles in *Peripatopsis* (p. 491) and which stains blue with Mallory, is the 'vitelline membrane' of Dendy (1902) and others, does not persist into embryonic life, as assumed by so many authors, and does not become the inner of two embryonic membranes.

(2) The inner cuticular embryonic membrane in *Peripatopsis* (pp. 491 and 494), which stains red with Mallory, and which may be a fertilization membrane, has not yet been seen in other genera, but as it does not persist long after segmentation is completed, and as no satisfactory account exists of the early development of any other genus, its presence may not be restricted to *Peripatopsis*. It may be thinner or thicker than the outer membrane.

(3) The outer chitinous embryonic membrane in *Peripatopsis*, staining blue with Mallory and secreted by the oviduct (pp. 492 and 494), has been seen by Sedgwick to persist until birth, but he questioned its origin, suggesting that it was either a vitelline membrane or produced by the egg follicles (1885). This membrane is presumably homologous with the single membrane surrounding the viviparous yolky embryos of *Peripatoides orientalis** and the yolkless *Paraperipatus novae-britanniae*.† It persists until birth in *Peripatoides orientalis* (Steel 1896). In *Paraperipatus novae-britanniae* (Willey 1898) it disappears when the body is fairly advanced in structure but the yolk sac not yet absorbed; in early stages it is very thick, the youngest uterine egg found of 100μ was invested by a membrane 75μ thick, a condition comparable to that here shown for *Peripatopsis sedgwicki*. Willey questions whether this membrane is a vitelline membrane or a chorion.

(4) A fourth membrane appears to be the outer 'chorion' or 'shell' of the oviparous yolky-egged forms which Dendy (1891) describes in *Ooperipatus oviparus* as 'almost chitinous-looking' and smooth when in the oviduct, but after it has passed through the vagina is 'exquisitely sculptured or embossed'. It is said to be secreted by the oviduct (Dendy 1902).

* = *Peripatus Leuckartii* Dendy (1902) and *Peripatus Leuckarti* var. *orientalis* Steel (1896).

† = *Peripatus* (*Paraperipatus*) *novae-britanniae* Willey (1898).

The interpretation of the membranes in the yolky-egged forms is less certain. In the viviparous *Peripatoides novae-zealandiae*,* Sheldon (1888) figures two membranes round the segmentation stages, a thin inner 'vitelline membrane' and a thick outer 'chorion'. She states that the chorion had to be removed before sectioning, and figures a vitelline membrane only round sections of advanced embryos. Surrounding the large yolky eggs of *Ooperipatus oviparus* and *O. viridimaculatus*, Dendy (1902) describes a thin inner membrane within the sculptured chorion, and he considers that these are homologous with the two membranes of *Peripatoides novae-zealandiae*. He also suggests that the inner membrane is homologous with the single membrane round the embryos of the viviparous yolk-less *P. orientalis*. If this were so, one would expect the thin inner membrane in *P. novae-zealandiae* and *Ooperipatus oviparus* to be chitinous and comparable with the outer membrane in *Peripatopsis*; and the outer thick shell of the yolky forms would then be absent from the yolkless viviparous types such as *Peripatopsis*, *Peripatoides orientalis* and *Paraperipatus novae-britanniae*. The persistence of the outer shell in the viviparous but yolky *P. novae-zealandiae* and its disappearance in the viviparous non-yolky forms is readily understandable if the latter have evolved from oviparous types, a conclusion strongly supported by the embryological details of *Peripatopsis*.

The two egg membranes of *Peripatopsis* bear resemblances to the two membranes described by Yonge (1938) and Mawson & Yonge (1938) round the eggs of Crustacea, but their order is inverted. A freely permeable chitinous membrane in *Homarus* and *Chirocephalus* lies within a rigid cuticular one of limited permeability. The chitinous membrane is secreted by the oviduct in these Crustacea and in *Peripatopsis*, and the function is protective in both. The cuticular membrane is produced by cement glands in *Homarus* and by uterine glands in *Chirocephalus*, but is probably produced by the egg itself in *Peripatopsis*. The different positions of this membrane in the two classes are correlated with their functions. In Crustacea the outer cuticular membrane attaches the egg to the parent and is protective in *Homarus*, while the thicker cuticular membrane in *Chirocephalus* enables the egg to resist desiccation. The outer position of the membrane is suitable for these purposes in the Crustacea, but in *Peripatopsis*, on the contrary, the rigid impermeable membrane is required by the non-coherent egg for the maintenance of its form in early stages, while the outer membrane is flexible, taking on the shape of the oviduct lumen and of the expanding embryo.

Mawson & Yonge (1938) have suggested that the complex explosive sperms of the Decapoda are necessary to penetrate the chitinous egg membrane. Both membranes in *Peripatopsis* would provide formidable barriers to the simple sperms of these animals, and here fertilization is presumably effected after the early egg membrane has been left behind in the ovary and before the two later ones are formed.

The unsegmented egg

The history of the ovum between leaving the ovarian follicles and the beginning of segmentation in the oviduct needs further study as insufficient stages have been examined. Sedgwick (1886, 1888) noted the 'sponge-like structure of the ovum', and 'an irregular central cavity' displayed by his technique, and the general appearance resembling a yolky egg 'from which the yolk has been almost completely dissolved out by some reagent'.

Several features indicate a secondary reduction in the quantity of yolk.

* = *Peripatus novae-zealandiae* (Sheldon 1888, 1889*a*, *b*).

Sheldon (1889*b*) correctly noted the 'remarkable difference in size between the oldest ovarian ovum and the youngest uterine one', and she describes a 'reticulum within the uterine eggs' and the appearance of a 'germinal vesicle' of dense protoplasm on one side containing the nucleus, shown here in figure 60, plate 37. This swelling of the egg (see also p. 489), and the later dilatation of the embryo at the end of segmentation (p. 491), both appear to indicate the past influence of yolk. An early gradual swelling accompanies the laying down of yolk in any egg, but a sudden subsequent dilatation is usually associated with the intake of water by that yolk. The yolk of all eggs needs to absorb water from within or without the egg before it can be fully utilized in metabolic processes (Needham 1942 and Manton 1934, pp. 185, 213). It is possible that the early dilatation of the egg in *Peripatopsis* indicates an ancestral absorption of water by a yolky egg which took place before the impervious inner membrane was laid down, the water being needful for the utilization of the yolk during segmentation stages, which, owing to the inner membrane, were not in free communication with their fluid surroundings. For further discussion of the dilatation of the yolk sac see p. 537.

It has been shown that the substance of the coherent egg breaks down into numerous spheres of protoplasm floating within the egg membrane, and that only one of these contains the nucleus. In the development of the yolky-egged crustacean *Nebalia bipes* it was found that the central nucleated mass of cytoplasm of the fertilized egg rises to the surface more rapidly than in many Crustacea (such as *Hemimysis*, etc.), and in so doing it fragments into a variable number of distinct portions. The nucleated first blastomere and several non-nucleated 'pseudo-blastomeres' are formed at the surface of the yolk (Manton 1934, text-figure 1*b*, p. 169). No particular significance was attached to this phenomenon in 1934, but it is associated presumably with abundance of yolk, as I know of no examples occurring in a non-yolky totally cleaving egg. The fragmentation of the cytoplasm of the egg of *Peripatopsis* appears to be essentially the same as the occurrence in *Nebalia*, the cytoplasmic spheres of the former being homologous with the 'pseudo-blastomeres' of the latter. If this is so, then the formation of the first blastomere and the cytoplasmic spheres in *Peripatopsis* indicates the existence of a yolky-egged ancestral stage. For further evidence of a secondarily yolkless condition see p. 535.

Previous work on Peripatopsis *and other Onychophora and the present account*

Professor Sedgwick's name will long be remembered for the abundance and excellence of his work on the Onychophora. It is to be regretted that with the imperfect methods at his disposal he happened to take for embryological study the South African *Peripatopsis capensis* and *P. balfouri* which present so many differences in early stages which he did not appreciate. As pointed out by Purcell (1906) 'the ova examined by Sedgwick. . . had in many cases been more or less strongly altered by external influences and no longer represented the normal course of development'. Purcell describes the 8-cell embryo for *P. balfouri* up to the stage here called *k* at the end of segmentation. He stresses the absence of anastomoses between the cytoplasmic spheres and describes their true form, but, like Sedgwick, he misinterpreted their nature (see below). Neither Sedgwick nor Purcell observed the dilatation of the embryo of *P. balfouri* or its transitory yolk sac, and they thus had no idea of the range of features shown by the genus. Bouvier made a few observations on the dimensions of the ova,

and on the form of the yolk sac and external embryonic features of *P. moseleyi* and *P. sedgwicki* which have been noted elsewhere.

Segmentation and the formation of endoderm

The differences between Sedgwick's description of segmentation in *P. capensis* and *P. balfouri* up to the 8-cell stage and the present account, illustrated by Sedgwick's (1885) figures 4 to 6, plate 31, and figures 16 and 62 to 64, plates 31, 36 and 37 here presented, do not merit a full discussion. Purcell has correctly criticized Sedgwick's figures, and more adequate methods have demonstrated that the egg does not segment either 'completely' or 'meroblastically' as Sedgwick states (1886, 1887), but that the nucleated first blastomere separates from the non-nucleated cytoplasmic and nutritive part of the egg, and cleaves independently from the latter.

Stages showing a saddle or disk of superficial blastomeres were observed by Sedgwick and Purcell, but both considered these cells to be ectodermal only, while in fact they give rise to all germ layers. Purcell as well as Sedgwick confused the non-nucleated cytoplasmic spheres of early stages (see Sedgwick 1885, figures 7, 8 and 10 *en.*, plate 31, and figures 62 to 67, plate 37 shown here) with the 'large vacuolated cells' derived from the edges of the saddle of blastomeres (see Sedgwick, figures 11, 12, 13 and 15 *en.*, plate 31 (figures 7, 11 and 12 are of *P. balfouri* and figures 8, 10, 13 and 15 are of *P. capensis*) and the figures on plates 37 and 39 of stages *i* to *k* of *P. balfouri* and figure 120, plate 41 of *P capensis* here shown).

Sedgwick further supposed that both these components represented endoderm; the large vacuolated cells in *P. capensis* do in fact give endoderm, but not in *P. balfouri* where the definitive endoderm does not arise until later (see p. 512). He accounted for the nuclei of his 'endodermal' cells by supposing that they arose *de novo* in the non-nucleated cytoplasmic spheres, so giving the nucleated large vacuolated cells which he correctly observed forming 'a ring-like mass applied all round the edge of the ectodermal patch' (1886). Although Sedgwick gives a long description and discussion of the development of 'endoderm' from the cytoplasmic spheres, he says (1887) of segmentation that, apart from the superficial blastomeres, 'the breaking up of the rest of the ovum into irregular masses cannot be regarded as a process in any way related to cleavage, inasmuch as the nucleus takes no part in it', and in a footnote, 'This process is probably identical with the formation of the non-nucleate yolk spheres found in many arthropoda.' The former conclusion is correct, but applies more forcibly to the facts of development here described than to the version arrived at by Sedgwick. Whether the cytoplasmic spheres represent yolk spheres is uncertain. They are clearly depleted of food material, and it has been suggested above (p. 529) that they may be homologous with the 'pseudo-blastomeres' of yolky forms such as *Nebalia*.

Sedgwick recognized the gastrula of *P. capensis*, although his methods did not show him the archenteron in early stages which arises in a typical manner (p. 501). He states that 'the gut of the gastrula arises from an enlargement and confluence of the vacuoles in the centre'...'of a much vacuolated mass of protoplasm', a conclusion which resulted from his technique. It has been shown that the open blastopore in *P. capensis* does not directly become the mouth-anus opening as Sedgwick supposed, although the latter may yet be found to arise on the site of the closed blastopore when embryos of exactly the appropriate age are examined. The mouth-anus arises after endoderm formation is completed.

Had Sedgwick been able to interpret the embryo of *P. balfouri* which he shows in figure 16, plate 31 (1885) and which corresponds to stage *k* shown here on plates 37 and 39 probably lacked a blastopore, and had he obtained stages *k* to *o* here shown, he could not have supposed that *P. capensis* and *P. balfouri* developed in a similar manner. Purcell (1906) with better fixation correctly noted the form and behaviour of the large vacuolated cells during late segmentation stages of *P. balfouri*, but like Sedgwick he assumed that these cells and the non-nucleated cytoplasmic spheres were the same thing, and with no further evidence considered them to be endodermal in nature, and stage *k* to be a 'solid gastrula'. The present account confirms the occurrence of a solid gastrula without archenteron, but the definitive endoderm which arises later was not seen by Purcell. Sedgwick's account of segmentation, of the origin of the endoderm, and his long account and observations on the syncytial nature of the embryo, the origin of nuclei *de novo*, and related topics will not be considered further. The significance of the differences in segmentation shown by the several species of *Peripatopsis* is considered on p. 536.

Blastoporal area or primitive streak and the formation of endoderm and mesoderm.

The earliest stages of immigration from a blastoporal area have not been recorded before. Very little or no endoderm arises from this region in *P. capensis*, and in *P. balfouri* all the endoderm has passed in from the blastoporal area before the first somite is formed. A later immigration of endoderm which Sedgwick thought he detected in these species can plainly be seen in *P. moseleyi* and *P. sedgwicki*, although it does not occur in the two former species.

Evans (1901) for *Eoperipatus* states that 'the mesoderm seems to be formed exclusively from the primitive streak', and 'that the endodermal elements are derived from the lips of the blastopore, with which the endodermal layer is continuous, and that they pass from that position through the outer layer of yolk'. A similar general account is given by Sheldon (1889*a*) for *Peripatoides novae-zealandiae*. The supposed evidence of invagination from the lips of the 'blastopore' (the mouth-anus) consists of (1) the continuity of the ectoderm and endoderm at this point, and (2) the fact that the endodermal layer is not complete when the first somite is formed and the mouth-anus is undivided. Both these features can be seen in *Peripatopsis* and are no proof that endoderm arises in this way. Evidence has been given above (p. 518) in support of the view that the mouth-anus is not concerned with endoderm formation, a conclusion confirmed by abnormal embryos which lack a mouth-anus yet possess a normal endodermal layer.

In the African and Australasian species all workers agree in finding mesodermal immigration from a primitive streak or blastoporal area. Sedgwick, working under the profound disadvantage of methods showing no cell boundaries, considered that the lips of the mouth-anus (called by him the blastopore) probably contributed cells to the mesodermal bands on either side (1887). This tentative suggestion has not been substantiated, and the mitoses present in the lips of the mouth-anus have been considered above (p. 519) to be concerned with the extensive growth of these lips, which form the mid-ventral part of the body besides the mouth and anus.

In the South American yolkless forms (Kennel 1884, 1886; Sclater 1888) information which modern methods would provide is lacking. However, the absence of a blastopore and

the formation of mesoderm and endoderm from a blastoporal area in *Epiperipatus imthurmi*, *E. torquatus* and *E. edwardsii** clearly resembles the condition in *Peripatopsis moseleyi* and *P. sedgwicki*, in spite of the great differences in form shown by the two types of embryos (see figure 7, p. 544), and does not contrast with the African forms as the original account of *P. capensis* suggested.

The mouth-anus and the origin of the mouth and anus

The large embryonic mouth-anus, which gives rise to the mouth and anus and ventral part of the body, has been seen in the African and Australasian Onychophora and figured by several workers, but only Sedgwick and Sheldon have attempted to study the origin of this structure. Balfour (1883) showed the enormous mouth-anus of *P. capensis* to divide into mouth and anus, and his figures were reproduced by Sedgwick (1885). Sedgwick identified the mouth-anus as a blastopore because he thought that the two structures were continuous in time, and Sheldon on no evidence assumed that the mouth-anus of *Peripatoides novae-zealandiae* was formed in a similar manner. The standard text-books and most subsequent workers have referred to this organ as a 'blastopore', but with no further justification.

It has been shown here that the mouth-anus in *Peripatopsis capensis* arises either before or simultaneously with the appearance of the immigration of mesoderm from a zone corresponding with the blastoporal area of other species of *Peripatopsis*, but in these species all possessing a large blastodermic vesicle, the mouth-anus is formed after immigration from the blastoporal area is well advanced. In *P. capensis* the blastopore does not directly become the mouth-anus, although future work may show that it almost does so, but in the species with large blastodermic vesicles the mouth-anus arises *de novo*, even, in *P. balfouri* where a transitory blastopore is formed. Kennel (1886) criticized Sedgwick's interpretation of his early stages with justification, and for *Epiperipatus edwardsii* he described, doubtless correctly, a blastoporal area to be present before any mouth or anus (1885), as in some species of *Peripatopsis*. From this area he records immigration of endoderm which fills the embryonic rudiment (figure 7 *S* to *U* here shown, p. 544); a gastric cavity then appears in this solid core of endoderm, and a mouth becomes differentiated by a secondary union of ectoderm and endoderm. The anus arises a little later just in front of the blastoporal area. There appears to be no reason to doubt these facts so clearly shown by the figures. *Epiperipatus* thus differs from the *Peripatopsis* species with large blastodermic vesicles only in the independent origin of the anus from the mouth, but this condition resembles the commoner state of affairs in other arthropods (see p. 543). The endoderm in *Epiperipatus*, being first a solid core while in *Peripatopsis* it lines a large hollow vesicle, are variations dependent upon the shape of the embryos.

The size of the mouth-anus varies widely in different species, as also does the stage at which it divides. In the non-yolky forms the very large size in *P. capensis* (figures 113 to 116, plate 41) is paralleled by the yolky *Eoperipatus weldoni* (Evans 1901), and in both it divides when somite formation is well advanced, three to five somites being formed, and the first somite having already become pre-oral in position. In *Peripatopsis balfouri* and *P. sedgwicki* the lumen of the mouth anus is narrower, and division occurs at an earlier stage (see p. 518). In *P. moseleyi* the mouth-anus remains closed until after its division, the lips parting when

* *Peripatus imthurmi* (Sclater 1888), *P. edwardsii* and *P. torquatus* (Kennel 1884, 1886).

about eighteen somites are formed. Kennel's figures of *E. edwardsii* show an initial mouth and anus also with closed lips.

Formation of mesodermal somites, germ cells and genital rudiment

Sedgwick's outline of the *formation of mesodermal somites* in *Peripatopsis capensis* and those of other workers are substantiated by the present more detailed account. It has been here shown that the segmentation of the mesodermal bands into paired hollow somites takes place evenly from before backwards, and that all somites arise in essentially the same manner. This simplicity is exceptional among both annelids and arthropods, where the majority exhibit the phenomenon of 'primary' and 'secondary' metamerism. The significance of this simplicity is discussed on p. 546.

The *segmentation of the head and body* has been studied as fully as possible in early stages in order to provide further data concerning the morphology and phylogeny of the head in Onychophora and Arthropoda. A consideration of head segmentation is given on pp. 555 and 556.

Sedgwick traced the *formation of the gonads* back to germ cells lying near the 16th to 20th somites in *P. capensis*. His claim that they arise in the endoderm at his stage *D* where most somites are formed need not be seriously considered, as his figures show that he did not have a technique which allowed a critical examination of these early stages. It has been here shown that germ cells in *P. capensis* are differentiated at a time when the cavities of the first pair of somites are appearing. In all species the germ cells lie outside the endodermal layer; they arise early, either from the undifferentiated mesoderm in *P. sedgwicki*, *P. capensis* and *P. balfouri*, or directly from the blastoporal area before any mesoderm is formed in *P. moseleyi*. This difference is probably one of degree and not of kind, depending upon the time at which the germ cells are first differentiated (p. 520).

The origin of the germ cells in *Peripatopsis* is comparable with the condition in other animals in which they arise early, even before the differentiation of the germ layers. For example, in the crustaceans *Hemimysis* (Manton 1928) and *Mesopodopsis* (Nair 1939), and in the annelid *Salmacina* (Malaquin 1924), the germ cells are extra-coelomic in origin, and later become invested by the coelomic wall. Similarly in the coelenterate *Gonothyraea* (Wulfert 1902) the germ cells arise in the planula, and in many other hydroids they first appear in the coenosarc, and migrate thence to the blastostyle and finally to the site of the gonad.

In contrast with the above, the germ cells may arise late, and at the site of formation of the gonad. In *Scolopendra* (Heymons 1901) and in the crustacean *Nebalia* (Manton 1934) germ cells are differentiated from the somite walls, and many coelenterates show germ cells first appearing on the site of the gonad.

As both types of germ cell origin, and also many intermediates, are to be found within the Coelenterata, Annelida, Arthropoda and possibly other phyla, the existence of an almost continuous 'germ line' associated with early differentiation of the germ cells cannot be of great significance. In arthropods an extra-coelomic origin of the germ cells is clearly a consequence of early differentiation of the germ cells, and is not necessarily associated with the genital rudiment being sometimes larger than the initial coelomic cavity with which it becomes associated.

Types of early development in the Onychophora

The various species of Onychophora in so far as they have been described, show three main types of early development dependent upon the size of the egg and the amount of yolk present.

(1) The South American species of *Epiperipatus* and *Macroperipatus* possess small uterine eggs of about 40μ, and they are viviparous.

(2) Larger eggs with very little or no yolk are found in many viviparous species; sizes of 100μ are found in the African *Opisthopatus cinctipes* (Purcell 1900) and Australasian *Paraperipatus novae-britanniae* (Willey 1898); sizes of 200 to 600μ occur in the several species of *Peripatopsis*; and of 370μ in the ovarian egg of *Peripatoides orientalis* (Dendy 1902).

(3) Heavily yolked eggs of still larger size occur in some Australasian species, 1·3 mm. in *Eoperipatus weldoni* (Evans 1901), and 1·5 mm. in *Peripatoides novae-zealandiae* (Sheldon 1888), both being viviparous; and the yolky eggs of several species of *Ooperipatus* reach 2 mm. (Dendy 1891, 1902). *O. paradoxus* is viviparous (Bouvier 1914), but the other species of this genus are oviparous.

The available details of segmentation of these types of eggs are limited.

Type 1. As far as is known from the work of Kennel (1884–6) on *Epiperipatus edwardsii* and *Macroperipatus torquatus* and that of Sclater (1888) on *E. imthurmi*, the small eggs undergo total cleavage. The imperfect methods used showed no cell boundaries, and the nuclei of an 8-cell stage are only separated by a small amount of cytoplasm and do not lie in one plane.

Type 2. Scarcely any information exists outside the genus *Peripatopsis*. Willey (1898) experienced extreme difficulties with the 75μ thick membrane of an egg but little longer than this in *Paraperipatus*, and his sections of early stages 'were all hopelessly collapsed'. There is no need to consider his further remarks on segmentation as he had no evidence for them. As the later stages of *Peripatopsis*, *Paraperipatus*, and perhaps *Peripatoides orientalis* show major features in common, *Peripatopsis* may be taken for the present as an example of this type of development.

Type 3. Little is known of the embryology of the several oviparous species of *Ooperipatus*, but the viviparous yolky *Peripatoides novae-zealandiae* has been studied by Sheldon (1888, 1889*a*). The account suffers from the usual defects due to the available methods of the time, and to incompleteness in the series of stages, but the work as far as it goes provides useful data. The blastomeres in early stages lie within the yolk (as they do in other yolky arthropods such as *Hemimysis*), and then rise to the surface to give a small disk of cells lying on the yolk (see Sheldon 1888, figure 24, plate 16, where thirteen cells compose the disk). This disk spreads gradually round the yolk to form a continuous blastoderm, just as occurs in many other yolky-egged animals. The details given of the origin of the endoderm and the process of gastrulation cannot be accepted (see pp. 518 and 531, but in a stage where the blastoderm covers only half the yolk Sheldon describes 'small masses of protoplasm in the centre of the egg, which masses sometimes contain nuclei'. These nuclei are shown on plate 25 (Sheldon 1889*a*) lying irregularly in the yolk mass, and appear to have nothing to do with the formation of the definitive endoderm, which takes place ventrally at the surface of the yolk. The origin and fate of these cells needs further study (see also p. 537). Evans (1901) obtained no stages of *Eoperipatus weldoni* earlier than one with a large mouth-anus (his 'blastopore') resembling that of *Peripatopsis capensis*, but disclosing the abundant yolk within the lips,

a 'primitive streak', the first somite and a rudimentary second and third somite are present on either side. The definitive endoderm is shown to spread round the yolk from the ventral side (its origin by invagination from the lips of the mouth-anus cannot be accepted, see pp. 518 and 531), absorbing the outer layer of yolk, but no nuclei lie in the central mass of yolk in early stages comparable to those occurring in *Peripatoides novae-zealandiae*.

Evidence for the view that Peripatopsis *is secondarily yolkless*

Sedgwick (1887) considered that the ovum of *P. capensis* 'has only recently lost its yolk, and that it may be compared to an ovum of the New Zealand form from which the yolk has been almost completely dissolved out'. Dendy (1902) has shown that 'the oviparous habit is very ancient, dating back at least to the Cretaceous epoch. The conclusions of Sedgwick and Sclater as to the loss of yolk in the eggs of certain viviparous species are thereby supported.' The reverse view was upheld by Willey (1898) and Bouvier (1904), who considered that there was insufficient evidence for the belief in the secondary loss of yolk in the viviparous yolkless forms. However, in 1916 Bouvier agreed that it was possible that the viviparous habit in *Ooperipatus paradoxus* has recently been acquired from oviparous ancestors, a conclusion advocated by Brues (1935), who has also some evidence that it occasionally lays eggs although normally producing live young. Sedgwick based his conclusions mainly on (1) the large size and non-yolky nature of the egg (see above p. 528) in *Peripatopsis*, and (2) the general similarity of development of the later stages of *P. capensis* and the yolky *Peripatoides novae-zealandiae*.

The present description of several species of *Peripatopsis* provides abundant support for Sedgwick's views, and it is impossible to account for the peculiarities of segmentation, germ-layer formation, and early development of *Peripatopsis* by supposing that a yolk-laden stage had never occurred. Features which appear to be correlated with an ancestral yolky condition are: (1) The nature of the egg membranes (see p. 527). (2) The size and early dilatation of the uterine egg and its general form (see p. 529). (3) The small 'first blastomere' occupying only a very small part of the space within the egg membrane, and separating from (4) the non-nucleated cytoplasmic spheres; these contain conspicuous food inclusions in *P. balfouri*, fewer in *P. capensis*, and none in *P. moseleyi* and *P. sedgwicki* (see pp. 496 and 529). (5) The discoidal segmentation taking place in one plane against the inner surface of the membrane (see p. 537). (6) The formation of a continuous blastoderm by the spreading of the disc of blastomeres until the edges meet, a blastopore being formed in *P. capensis* and *P. balfouri*, but not in *P. sedgwicki* and *P. moseleyi* (see p. 536). (7) The breakdown and absorption of the cytoplasmic spheres, which may represent a nutritive part of the egg (see pp. 496 and 530). (8) The second dilatation which follows segmentation may indicate a recapitulation of the behaviour of a yolk-filled yolk sac (see pp. 491 and 537). (9) The presence of a blastoporal area or primitive streak, a feature noted by Sedgwick (1884) to be generally associated with yolky embryonic developments and to be absent from animals with early larvae. (10) The endoderm arising from the edges of the disk of blastomeres in *P. capensis* (pp. 501 and 530), and (11) the replacement of this endoderm by cells immigrating inwards from the blastoporal area or primitive streak in several species (see pp. 501, 510 and 512). (12) The nature of the yolk sac although this organ contains no yolk (see p. 537).

The opposite view, quoted above, concerning the ancestry of the viviparous non-yolky Onychophora of type 2 cannot be seriously entertained. Willey (1898) states that 'there is no reason whatever to suppose that there has been a secondary loss of yolk' in *P. capensis* and in *Paraperipatus novae-britanniae*, but he obtained no clear details of either segmentation of germ-layer formation, and had in fact no data with which to uphold his views. Bouvier had no embryological evidence of his own. His views are discussed on p. 538.

Differences in segmentation, germ-layer formation, yolk sac, etc., occurring in the several species of Peripatopsis, *and their bearing upon evolution within the Onychophora*

The state of the literature on onychophoran development has given the impression that there is wide variation in ontogeny within the group, and 'in extreme cases the assembling of the germ layers seems to be almost haphazard' and 'it would appear that the manner of development has little significance' (Snodgrass 1938). I am in disagreement with both these statements. On the contrary, when facts are sifted from fiction, there is extraordinary uniformity in the essential processes of development, in spite of the enormous variations in the amounts of yolk present in the eggs of different species, and the manner of development has considerable significance.

Segmentation and germ-layer formation

A basic similarity in development is shown by the species of *Peripatopsis*, but the variations in segmentation and germ-layer formation found within the same genus are remarkable. Early segmentation appears to be essentially similar in the four described species. In later stages large vacuolated cells are formed from the edges of the disk of blastomeres in *P. balfouri* and *P. capensis* but not in *P. moseleyi* or *P. sedgwicki*. In the two former species the vacuolated cells become internal in a comparable manner, in *P. capensis* forming a hollow gastrula with archenteron and blastopore, while in *P. balfouri* a solid gastrula results. A clear blastopore is present in *P. capensis*, in *P. balfouri* the homologue of a blastopore is very early obliterated, and in *P. sedgwicki* and *P. moseleyi* no blastopore is formed. The large vacuolated cells in *P. capensis* give rise to the endoderm, little or none being added later from a primitive groove (blastoporal area). The vacuolated cells in *P. balfouri* degenerate and the endoderm arises later from the blastoporal area. In *P. moseleyi* and *P. sedgwicki* endoderm arises as in *P. balfouri*, but its immigration is a more prolonged process. *P. balfouri* thus provides an intermediate condition between *P. capensis* on the one hand and *P. moseleyi* and *P. sedgwicki* on the other. Lastly, a 'giant cell' of glandular function is present in *P. balfouri* and *P. capensis* but not in the other species.

The absence of a blastopore in *P. moseleyi* and *P. sedgwicki* appears to be associated with the absence of vacuolated cells and the mechanically simple method of completion of the blastodermic vesicle by the formation of first a saddle of blastomeres and then a cylinder with open ends.* The gastrula of *P. capensis* with its two germ layers and a blastopore must be homologous with the blastodermic vesicle of *P. moseleyi* and *P. sedgwicki* which lacks both inner layer and a blastopore, and bears a superficial resemblance to a blastula. Had it not been for the intermediate condition shown by *P. balfouri* the blastodermic vesicle might have been regarded as representing a blastula. A difficulty in interpreting the blastodermic vesicle

* It is possible that this method is not constant and that variations may occur.

in *Peripatopsis* is reminiscent of the controversies concerning the interpretation of the 'blastoderm stage' of insect ontogeny which has been regarded as a blastula by some workers and a post-gastrula by others (see Eastham 1930, etc.).

The blastoporal area or primitive streak is in all species a clearly homologous feature. However, it gives rise to mesoderm only (see p. 515) in *P. capensis* and to endoderm as well in the other three species. The origin of the blastoporal area is in immediate approximation to the posterior border of the mouth-anus, whether the latter opens early (*P. capensis*) or late (*P. moseleyi*), and in the absence of a blastodermic vesicle in *P. capensis*, the first immigration of mesoderm takes place from the actual lip of the mouth-anus (figure 123, plate 41). In all species the mouth-anus and the blastoporal area very soon become separated. If the mouth-anus in *P. capensis* should prove to be formed upon the site of the blastopore, then Sedgwick's suggestion that the mouth-anus and the primitive streak represent parts of an elongated blastopore may be roughly true, although in *P. balfouri* the two former structures are at first far apart (figure 74, plate 37). It is noteworthy that whether the mouth-anus arises from the closed blastopore, as is probably the case in *P. capensis*, or whether it arises *de novo*, as occurs in the other species, they all show the endodermal layer to be established *before* the mouth-anus is formed.

When the ancestors of the Onychophora possessed a primitively small egg before abundant yolk had been evolved, cleavage probably formed a rounded embryo during segmentation, and endoderm would be developed from cells at the vegetal pole. When the increase in the yolk led to the formation of a single flat disk of blastomeres lying upon the yolk, the original vegetal pole cells would lie at the periphery of the disk, the position in which the large vacuolated cells first appear. It is noteworthy that in the Mollusca a similar response to the presence of yolk is found, and the Cephalopods show endoderm first appearing at the periphery of a disk of blastomeres lying on the yolk. *P. capensis* may thus show a derivative of the method of endoderm formation of a non-yolky ancestor. In the other species here described the mechanical difficulties attendant upon the large amount of yolk they once possessed may have led to the abandonment of the primitive method of endoderm formation (from the large vacuolated cells), which became replaced by one of immigration from the blastoporal area after the blastodermic vesicle was completed, or even before this event as is suggested by the work of Evans (1901) for *Eoperipatus* (the vacuolated cells either degenerating or not being formed). A secondary reduction in the amount of yolk would then give the conditions as we see them to-day. It is not unreasonable to suggest that there may have been considerable specific differences in development at a yolky stage. The little that is known of the present-day yolky Onychophora shows them to differ one from another; *Peripatoides novae-zealandiae* shows cells lying in the yolk in early stages, while *Eoperipatus weldoni* does not. Thus a consideration of endoderm formation appears to show some of the responses of the Onychophora to an increasing amount of yolk in the egg. The dilatations of the embryo and the form of the yolk sac, however, give us data upon the reverse process (see below).

The yolk sac

A yolk sac full of yolk is present in the oviparous species of Onychophora and in some viviparous ones (*Eoperipatus novae-zealandiae*). In the yolkless types the yolk sac is very large

in *Paraperipatus novae-britanniae*, where it extends beyond both anterior and posterior ends of the definitive embryo when all the segments are well established (Willey 1898). It is a little smaller in *Peripatopsis sedgwicki*, where it is antero-dorsal in position (figures 12 to 15, plate 31). In *P. moseleyi* it is relatively smaller still, lying over much of the dorsal region with but a small anterior extension (figures 35, 36, plate 33). In *P. balfouri* a large but transitory yolk sac is formed (figures 72 and 76, plate 37), and a special mechanism appears to have been evolved for its rapid elimination. Finally, in *P. capensis* the embryo fails to dilate although the egg membrane does so (figure 2), and so no large yolk sac is formed (figures 2 and 113 to 118, plate 41). The conclusion that *P. capensis* is further removed from its yolky ancestors in this respect than are the other species of *Peripatopsis* here described is supported by the behaviour of the egg membrane. Had dilatation been primitively absent the size changes of this structure (pp. 491 and 493), which occur exactly as in *P. balfouri*, would be inexplicable (see figure 7, p. 544, where the egg membrane is dotted and the limits of the embryo are shown by solid lines).

The possible usefulness to the embryo of a large but yolkless yolk sac serving as an absorptive organ has suggested a ready explanation of the great development of this structure. Thus Willey (1898) and Bouvier (1905) describe it as a trophic organ or vesicle, with a secondary protective function as a water cushion (Willey). This explanation, however, appears to be untenable. In the species with a large empty yolk sac development proceeds for many months after its absorption, and during this period the length of the embryo may increase 3 to 4 times. In *P. balfouri* the yolk sac, although large has but a transitory existence, and in *P. capensis* it is not formed at all. As the entire development of a considerable part of it can take place in the absence of this organ the yolk sac can hardly have an essentially nutritive function. It is more likely that the great size of the yolk sac represents a recapitulation of an ancestral dilatation of the yolk sac when it was filled with yolk. In the ancestral yolky embryos water must have been taken in for utilization by the yolk (see p. 529), and it appears that, although there is no longer any yolk, water is still absorbed and the properties of the membranes have not changed.

Bouvier (1904, 1905 and 1907) put forward the view that the large yolk sac (trophic vesicle) of *P. sedgwicki* is a primitive feature in the genus, and that it is coupled with the occurrence of small eggs which are considered to be the least removed from his supposed small-egged ancestor. He suggests that evolution has proceeded by the enlargement of the eggs and a reduction of the yolk sac, a first stage being shown by *P. moseleyi*, and complete atrophy of the yolk sac being arrived at by *P. capensis* with the largest eggs. He further considers that *P. sedgwicki* most closely approaches *Paraperipatus novae-britanniae*, but that it is less primitive, as its yolk sac is smaller and the eggs larger (pp. 534 and 537) than they are in *Paraperipatus*. However, he also suggests that this evolutionary line is primarily non-yolky, and is distinct in this respect from the heavily yolked Australasian forms. He considers that there are three separate evolutionary lines within the Onychophora descending from a hypothetical ancestral type with small eggs and a free aquatic development: (1) the small-egged South American species, specializing in the formation of something resembling placental nutrition, (2) *Paraperipatus* from New Britain and the South African species of *Peripatopsis*, non-yolky types which elaborate a 'trophic vesicle' and then secondarily revert to 'direct nutrition' from the parent in the more specialized forms (e.g. *P. capensis*),

and (3) the Australasian forms with yolky eggs, which specialize by increasing the quantity of yolk and the size of the eggs.

The present account supports Bouvier's suggestion that the reduction of the yolk sac in *P. capensis* is secondary, but it does not support his views concerning the supposed elaboration of a trophic vesicle without the intervention of yolk, and thus of necessity constituting an independent evolutionary line from the yolky-egged Australasian forms. Neither does a small egg, such as that of *P. sedgwicki*, appear to be necessarily primitive if the eggs in the *Peripatopsis* species have descended from yolky types. The four species of *Peripatopsis* here described would not be expected to form an evolutionary series in the sense of preserving evolutionary stages along one line at progressive intervals. They each have advanced to different degrees in certain characters. *P. capensis* may be the most advanced in its complete elimination of the yolk sac and may at the same time show the more primitive method of endoderm formation, while *P. sedgwicki* may show the least reduction of yolk sac, but, presumably at a yolky stage, it has eliminated the large vacuolated cells (see p. 537) and developed its endoderm from a primitive streak. *P. moseleyi* has changed its endoderm in a similar manner but has further reduced the yolk sac, and *P. balfouri* shows an intermediate condition in both respects.

The South American species may prove to be far less different from other Onychophora in their embryology than Bouvier and others have supposed. He regarded them as primarily yolkless, and as representing an independent adaptation to land life (1904), but Sedgwick (1887) supposed that they have 'been derived from the large-yolked ovum of some remote ancestor', although he had no evidence of this.

If the embryonic rudiment of the South American *Epiperipatus edwardsii* (Kennel 1884, plates 5 and 6) and *E. imthurmi* (Sclater 1888, figures 11 to 14, plate 24) and figure 7*S* to *U*, p. 544, be compared with the embryos of *Peripatopsis balfouri* and *P. capensis* after they have absorbed the bladder-like yolk sac and wild *P. capensis* (figures 78 to 83 and 113 to 118, plates 38 and 41 and figure 7*L* to *R*) a striking similarity is seen. In both the germinal bands and definitive embryonic tissues form most of the embryonic rudiment, and in both a narrow dorsal and ventral tract of thin tissue appears later, representing a reduced dorsal and ventral wall of the yolk sac as seen in *P. moseleyi*, etc. In the South American species a connexion from the dorsal region opposite the second pair of somites unites the embryonic rudiment with the surrounding vesicle. In position this 'Nabelstrang' of Kennel corresponds with the antero-dorsal attachment of the yolk sac in the stages of *P. sedgwicki* shown in figures 14 and 15, plate 31. In the absence of full information, it is tempting to suggest a possible homology between the outer 'vesicle' of the South American forms and the yolk sac of *Peripatopsis*, the embryonic rudiment of the South American species having become internal to the vesicle while lying primitively on its outer surface in *Peripatopsis*, and the rudiment being united by a similar stalk to the vesicle in both. Moreover, the earlier stages of the South American *Peripatus*, when the undifferentiated embryonic rudiment forms a local thickening bulging inwards from a large vesicle (Kennel 1884, figures 9, 10, 53, etc., plates 5 and 8), bear a superficial similarity to the stages of *Peripatopsis* shown in figures 48 and 49, plate 35 (the whole blastodermic vesicle has not been figured here). It is possible that the development of the South American species may be found to resemble basically that of other genera, but to represent a condition farthest removed from a yolky oviparous stage and to show specializa-

tions of their own. Thus it may be that the three separate evolutionary lines within the Onychophora envisaged by Bouvier may be far less distinct than he supposed, and all may have passed through a common yolky-egged state.

The multiphase theory of gastrulation

Roonwal's presentation of the multiphase theory of gastrulation in Arthropoda (1939) calls for some comment. I consider that no service is rendered by forcing a complicated theoretical interpretation and terminology, elaborated initially for insects (Roonwal 1936), on to simple developmental processes, as is done in the application of this theory to the Crustacea and Onychophora (incidentally Roonwal considers only the Malacostraca under the heading of Crustacea). Where similarities exist between different Crustacea, or between Crustacea and other Arthropoda, they are more forcefully and usefully expressed directly. I find Roonwal's interpretation of my accounts of crustacean development almost unintelligible, and I cannot agree with either of the statements in the following quotation: 'Crustacean endoderm shows a clear quadruple nature, and the other two layers show this nature less clearly.' I do not place any theoretical significance on the minor differences referred to by Roonwal in my accounts of mesoderm development in *Hemimysis* and *Nebalia*. The head band mesoderm of the Crustacea clearly represents part of the mesodermal bands of the Onychophora, and presumably of the ancestral arthropod (see p. 552), but supplies the three naupliar segments only. The origin of the teloblastic trunk mesoderm and the extra-blastoporal pre-antennulary mesoderm are doubtless innovations of the Malacostraca. The telson mesoderm, shortly described in *Nebalia*, and awaiting fuller description in other Malacostraca, in my opinion does not merit Roonwal's interpretation as a fourth method of mesoderm formation. It arises from the mesendodermal mass as do the paired head bands, and probably represents cells of the posterior unsegmented end of the mesodermal bands of an ancestral arthropod or annelid supplying the unsegmented posterior extremity of the body.

Roonwal's remarks (1939) on the Onychophora are truly surprising. He applies his theory to the literature on the group, and claims to find not only two phases of gastrulation, but 'a third phase is also faintly recognizable'; and further, that the 'quadruple nature of the germ layers in the Onychophora is clear in the case of the endoderm, but less so for the other two layers'. For his evidence he takes two examples of 'superficially cleaving eggs', *Peripatoides novae-zealandiae* and *Eoperipatus weldoni*, although the cleavage of the latter has never been seen, and neither of them has been adequately described. The youngest *Eoperipatus* examined (Evans 1901) already had a mouth-anus, blastoporal area, and three pairs of somites differentiated, and so provides no evidence at all of segmentation and early development; and the account of *Peripatoides novae-zealandiae* is fragmentary, and in part almost certainly erroneous (see above p. 531), and as stated by Snodgrass (1938) 'in this species the manner of germ layer formation has not been definitely determined'. Roonwal finds his theory to be applicable to the Onychophora, although a continuous series of stages showing segmentation and germ-layer formation was unknown before the present work. His evidence for the statements quoted above is negligible; no faith can be placed on theoretical work raised on non-existent foundations, and the application of this theory to

the Crustacea and Onychophora is both confusing and misleading. It is not proposed to discuss the multiphase theory of gastrulation further in connexion with the Onychophora as it would not lead to anything of constructive value.

Discussion Part 2. General

Blastopore, mouth-anus and germinal disk in Annelida and Arthropoda

Sedgwick wrote much concerning the blastopore and the primitive streak (1884, etc.), and he states concerning the primitive streak: 'I agree with the current view as to its nature as a rudiment of the blastopore'. It has been shown here that in *P. capensis* the primitive streak arises in the posterior lip of the open mouth-anus, and if the latter arises, as is probable, from a reopening of the blastopore (which is present in this species but not in most known Onychophora), then both mouth-anus and primitive streak may be regarded as derivatives of a blastopore.

Two important likenesses between the development of the Annelida and the Onychophora have been supposed to lie in (1) an 'elongation of the blastopore on the ventral surface... followed by the closure of its median part...leaving the persistent oral and anal apertures at the two extremities' (Snodgrass 1938), both are features described by Balfour (1883) and elaborated by Sedgwick (1884, etc.); and (2) 'the forward growth of the mesoderm as bands of cells generated from a proliferating area behind the blastopore' (Snodgrass 1938). Neither of these quotations can be accepted as they stand. A consideration of (1) is given below and (2) on p. 546. In view of the wide variations in the features just mentioned shown by the Annelida, the Onychophora and the other Arthropoda a brief review seems called for.

In the Annelida the simplest and possibly the most primitive method of gastrulation is by invagination of the vegetal pole of the blastula, resulting in a wide blastopore (figure 6*B*), such as seen in many polychaetes with little yolk in the egg and with early swimming stages. In polychaetes such as *Pomatoceros*, *Podarke*, *Nereis*, etc., the blastopore divides to form the mouth and anus of the trochosphere larva (figure 6*D* 1 and *E* 1) as noted by Sedgwick (1884). This is usually regarded as the most primitive condition within the group. The blastopore is here concerned with two processes: (1) the putting in place of the endoderm, and (2) the formation of the mouth and anus. However, these two functions are not necessarily associated with the same organ, and can be partially or completely dissociated in some annelids and arthropods. In many polychaetes the blastopore closes posteriorly, its anterior part alone remaining to form the mouth, and the anus then arises later from a new intucking, which may be situated approximately over the site of the obliterated blastopore (figure 6*D* 2 to *E* 2); and in others the whole blastopore is obliterated and a new anus and a new mouth are formed, as in the more yolky *Capitella*, etc.

In the more heavily yolked Arthropoda cleavage is various, but it frequently results, by different means, in a similar end stage where a superficial layer of blastomeres (the blastoderm) surrounds a central mass of yolk (figure 6*G*), the yolk taking the place of the blastocoele of the polychaete blastula (figure 6*A*). Invaginate gastrulation usually does not take place, although examples of it can be found, as in *Astacus* (Reichenbach 1886) and *Anaspides* (Hickman 1937). In *Astacus* a small invagination and a typical blastopore are

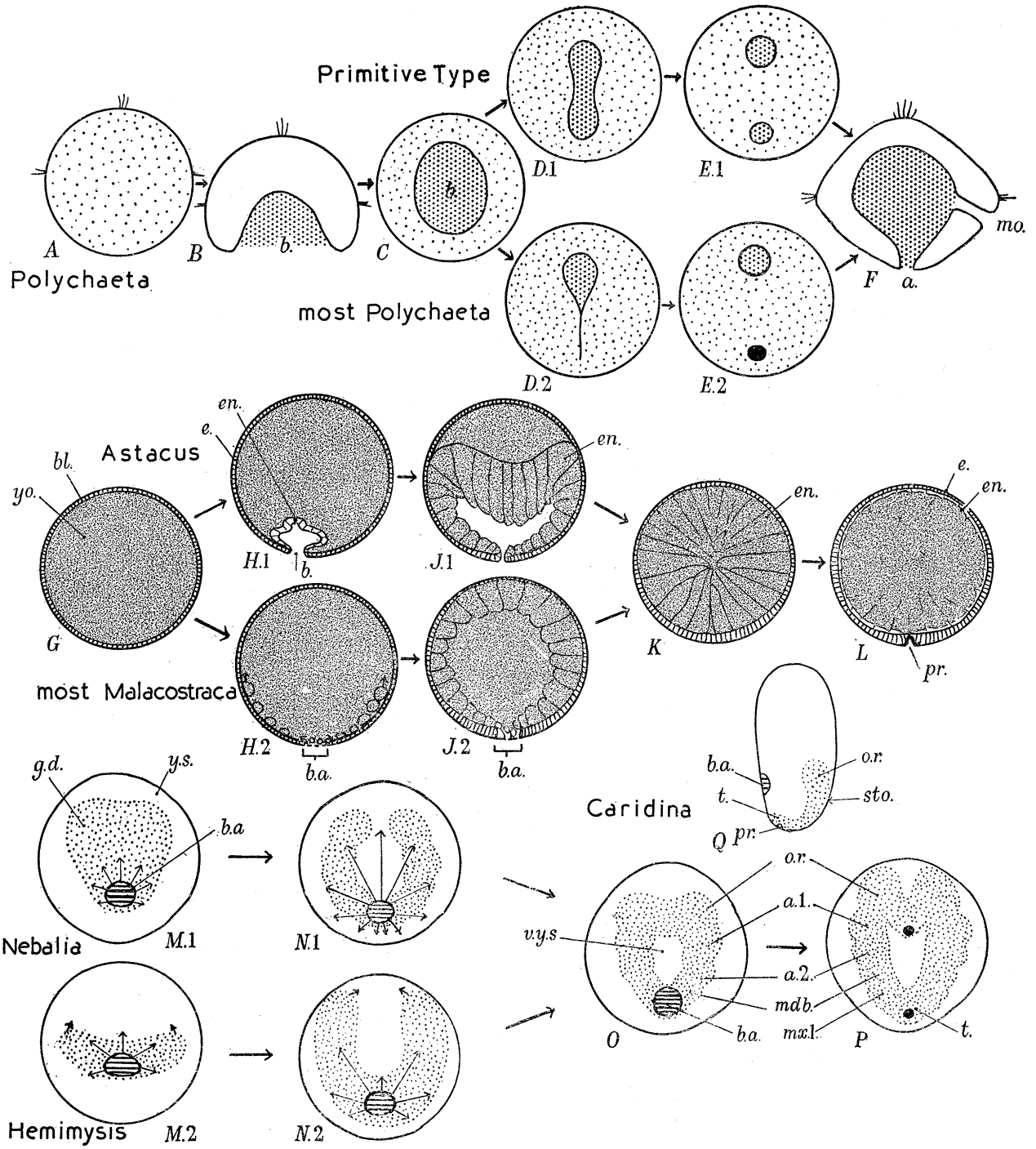

FIGURE 6. Diagrams of early stages of Polychaeta, Crustacea and Onychophora in either surface view or optical sagittal section, showing the relationships of the blastopore, the blastoporal area, the mouth, the anus, the definitive embryonic tissue and the yolk sac. The blastopore is marked *b.*, the blastoporal area ***b.a.*** is cross-hatched, the mouth and anus when derived from the blastopore are mechanically stippled, but when formed independently they are shown in black. The definitive embryonic tissue is stippled and the yolk sac is left white. The arrows radiating from the blastoporal area indicate the extent of the endodermal immigration, but when endoderm lines the whole blastoderm the arrows are omitted. Yolk, either free or enclosed in endodermal cells, is mechanically tinted. *A*. Polychaete blastula. *B*. Polychaete blastula invaginating. *C*. Polar

formed (figure 6*H*1); the invaginating epithelial cells absorb yolk and swell (*J*1), and most of the cytoplasm and the nucleus pass to a superficial position while the inner parts of the cell walls break down (*K*), so leaving a double-layered embryo with the yolk surrounded by endoderm (*L*).* In other yolky arthropods, such as the Malacostraca, a coherent endodermal epithelium usually does not invaginate, but from an area corresponding with the blastopore of *Astacus*, and therefore termed the blastoporal area (Manton 1828, 1934; Nair 1939, 1941), cells immigrate inwards separately, and travel round the yolk towards the dorsal side, absorbing it as they go, and thus increasing in size (*H*2); when they are sufficiently numerous they form a continuous layer (*J*2) by which most of the yolk becomes absorbed, then their inner parts break down, and the result is the same as before (*K* and *L*).* In *Astacus* the proctodoeum arises on the site of the vanished blastopore (*L*) and in *Hemimysis*, *Mesopodopsis* and *Nebalia* it arises approximately in the same position, however, in the decapod *Caridina laevis*, Nair (in the Press) has shown that the proctodoeum (*pr.*) arises at a considerable distance morphologically anterior to the blastoporal area (*b.a.*, *Q*).† The stomodoeum is a new invagination in all these Crustacea, lying far anterior to the blastopore or blastoporal area (figure 6*P* and *Q*); it is not formed until after the endoderm has reached this situation (figure 6 *M* to *P*, the arrows indicate the extent of the spreading endoderm, which lies

* In either case this primary endoderm may be replaced to various extents by surrounding epithelia which give permanent enteric structures. These arise from either endodermal plates, the ectodermal proctodoeum, or the mesodermal liver lobe rudiments, but we are not here concerned with these developments.

† My thanks are due to Professor K. Bhaskaran Nair for kindly allowing me to quote his unpublished data for *Caridina*, and for the use of the diagram shown in figure 6 *Q*, the original of which he prepared for me.

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view of the blastopore at a stage later than *B*. *D*1 and *E*1. View from the vegetal pole of the blastopore dividing to give mouth and anus, as in *Pomatoceros* and others. *D*2 and *E*2. View from the vegetal pole of the blastopore forming the mouth only, an anus appearing from a new intucking, as in most polychaetes. *F*. Polychaete trochosphere larva. *G*. Yolk-filled blastula stage of most Malacostraca. *H*1 and *J*1. Invaginate gastrulation of *Astacus*, the invaginating endodermal epithelium progressively absorbs the yolk. *H*2 and *J*2. Immigration of endoderm from a blastoporal area, the endodermal cells spread round the yolk separately, absorbing it progressively, as in most Malacostraca. *K*. The yolk is now absorbed by the endodermal cells, their nuclei are superficial, and their inner parts are breaking down. The blastoporal area is obliterated. *L*. The breakdown of the inner parts of the endodermal cells leaves the yolk enclosed by a thin endodermal epithelium, *en*. The anus is formed from a new intucking *pr*. *M*1 and *N*1. Germinal disks of *Nebalia bipes* showing the origin of the U-shaped germinal band by division of a single pear-shaped area. The naupliar segments are established in *N*1. *M*2 and *N*2. Germinal disk of *Hemimysis lamornae* showing the origin of the U-shaped germinal band by the approximation of the arms of an almost transverse area. *O*. Arms of the germinal band united anteriorly, leaving a thin-walled ventral zone (*v.y.s.*) between the future labrum and the blastoporal area, bounded laterally by the germinal bands. This thin zone corresponds with the ventral wall of the yolk sac in the Onychophora (figure 7). *P*. Later stage showing the independent origin of the stomodoeum and proctodoeum. When endoderm formation is a prolonged process, as in *Hemimysis*, the proctodoeum arise long after the stomodoeum, and the germinal bands are considerably longer than shown here by the time the proctodoeum appears. *Q*. Lateral view of embryo of *Caridina laevis*, by courtesy of Professor Nair. The teloblastic area *t*. surrounds the proctodoeum, and both lie a considerable distance morphologically anterior to the blastoporal area, and not in approximately the same position as in *P*. For further description see text.

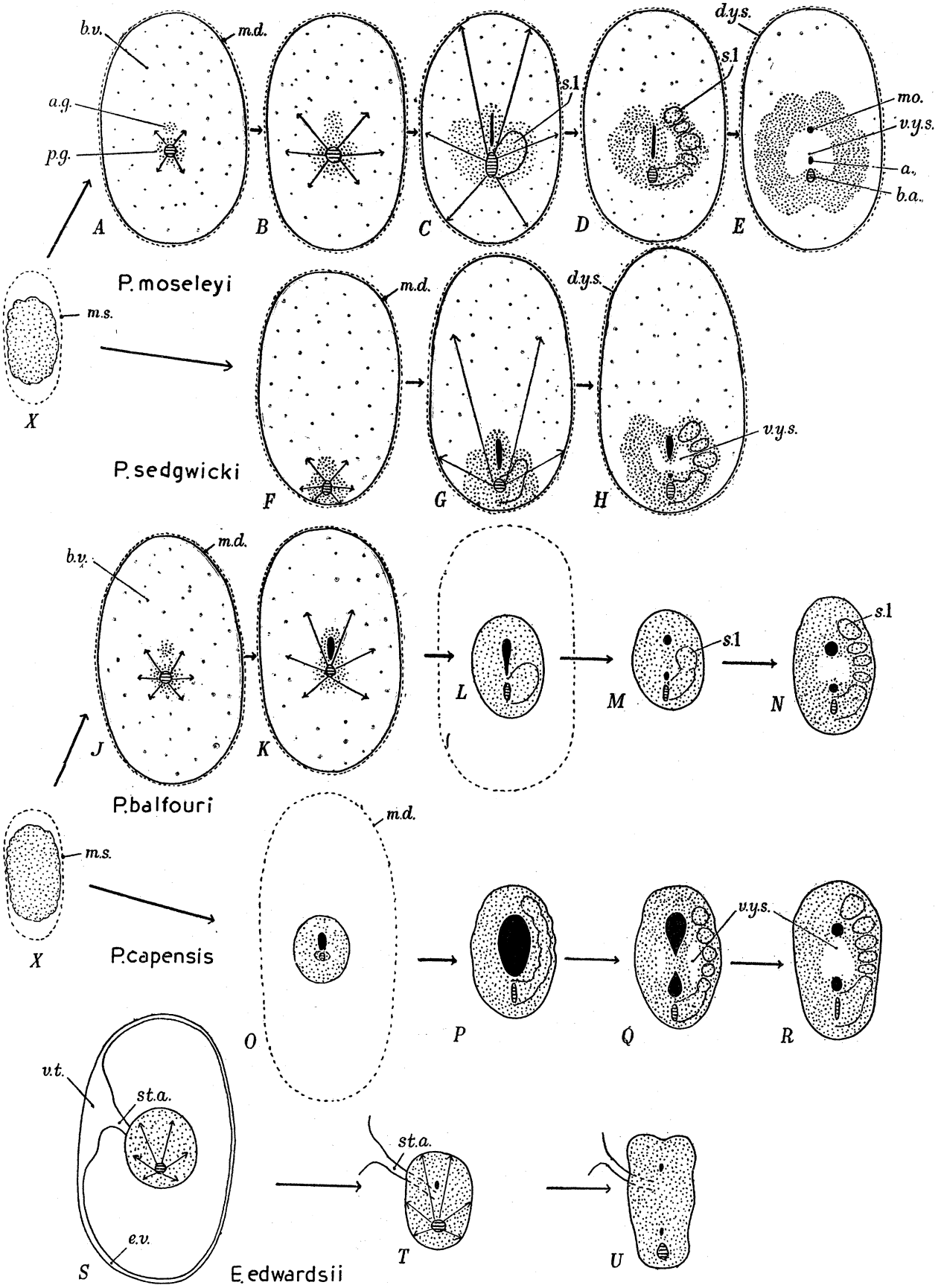

FIGURE 7. For legend see opposite.

everywhere in *O* and *P*). Thus the two attributes of the blastopore mentioned above for polychaetes such as *Pomatoceros* are dissociated in the Malacostraca, and markedly so in *Caridina*, where even the site of the blastoporal area is not used in the formation of the proctodoeum.

Peripatopsis capensis probably most closely approaches the primitive polychaete type, as the blastopore, after brief closure, probably reopens to form the mouth-anus which later divides, and the primitive streak arising in the posterior lip of the mouth-anus and spreading posteriorly, corresponds in position with that of the 4*d* blastomere of a polychaete (figure 7 *O* to *R*). In the species of *Peripatopsis* with large blastodermic vesicles and in the South American species of *Epiperipatus* either no blastopore or only a very transitory one (*Peripatopsis balfouri*) is formed; the blastoporal area in these forms corresponds to the primitive streak of *P. capensis*, and so is presumably homologous with at least part of a blastopore. Anterior to the blastoporal area arises the mouth-anus in these species of *Peripatopsis* and the separate mouth and anus in *Epiperipatus* (figure 7). It is clear that most of the known Onychophora fall into line with the Arthropoda in the dissociation of the mouth and anus from the blastoporal area and contrast with the majority of Polychaeta. The fission of the single initial mouth-anus to form a mouth and anus has not been found in the other Arthropoda, but the separate origins of mouth and anus in *Epiperipatus*, both of which lie anterior to the blastoporal area, corresponds exactly with the arthropod *Caridina* and doubtless many others (figure 6 *Q* and figure 7 *S* to *U*).

A different interpretation of a blastopore in the Malacostraca has been presented by Sollaud (1933) which cannot be maintained in conjunction with the present considerations. In figure 6 *M* to *P* and figure 7 are shown the germinal disks of two types of Malacostraca and of the Onychophora. The thickened tissue of the germinal band is lightly stippled, the regions comprising the yolk sac or its equivalent remain white, and the blastoporal area is horizontally ruled. In *Hemimysis* the arms of the V-shaped germinal bands diverge widely (figure 6 *M*2) and later approximate anteriorly and finally unite (*N*2 and *O*). In *Nebalia*, on the contrary, the disk is at first pear-shaped (*M*1), then it divides anteriorly becoming

FIGURE 7. Diagrams of the embryos of different species of non-yolky Onychophora. Conventions as in figure 3, the egg membranes are shown by dotted lines, and the limits of the embryos by solid lines, and mesodermal bands and somites are indicated by outlines on the right sides of the figures. The blastodermic vesicle (*t.bl.*) is relatively larger than is here shown in figures *A* to *K*. *A* to *E*. *Peripatopsis moseleyi*, the mouth-anus in *C* and *D* has divided to form the closed mouth and anus in *E*. *F* to *H*. *P. sedgwicki*, the open mouth-anus in *G* early divides to form an open mouth and anus in *H*. *J* to *K*. Early stages of *P. balfouri* with dilated blastodermic vesicle. The mouth is closed by a fine sheet of cytoplasm. *L* to *N*. Shows the development in *P. balfouri* after the sudden contraction of the vesicle, and early division of an open mouth-anus. *O* to *R*. *P. capensis* showing formation of a compact embryo directly from stage *X*, the membrane alone dilating. The dilated membrane (*m.d.*) is omitted from figures *P* to *R* in which an enormous open mouth-anus divides to form mouth and anus. *S* to *U*. *Peripatus edwardsi* (after Kennel 1884). *S*. Definitive embryo within the embryonic vesicle (*e.v.*) to which it is attached by a stalk (*st.a.*) uniting it with the vesicular thickening (*v.t.*). *T*. Later stage where an independent mouth has been formed. *U*. Later stage where the anus has been formed independently from the mouth and the blastoporal area. *X*. Segmentation stages in which the embryonic tissue does not yet fill the undilated egg membranes (*m.s.*).

V-shaped (*N*1), and later the arms of the V approximate and unite (*O*). Intermediate types exist in which the arms of the V are initially less divergent than in *Hemimysis*. The labrum is formed after the bands meet, and mid-ventrally between the labral rudiment and the blastoporal area lies a zone with thinner ectoderm than on the germinal bands and where mesoderm is absent (*O*, *v.y.s.*). This zone Sollaud calls 'l'aire blastoporique primaire', from which he considers a limited amount of endoderm may arise.* The blastoporal area he calls 'l'aire blastoporique secondaire' which he admits forms most of the endoderm and all the mesoderm. He considers that 'l'aire blastoporique primaire'... 'correspond manifestement au bords du large blastopore primitif des Polychètes'. Now if *O* and *P* are compared with the Onychophora (figure 7), it is seen that the four species of *Peripatopsis* show a thin zone, described above as the ventral wall of the yolk sac, appearing on either side of the mouth-anus in *D* and on either side of the separate mouth and anus in *Q* when the mesodermal bands have spread forwards and started to bow outwards. Further growth leads to fusion of these areas mid-ventrally between the mouth and anus (*E*, *H*, *N* and *R*). It seems clear that the general form of the germinal disks of *P. moseleyi* (*C*) and *P. sedgwicki* (*G*) corresponds with that of *Nebalia* (*M*1), and those of *P. moseleyi* (*E*), *P. sedgwicki* (*H*), *P. balfouri* (*N*) and *P. capensis* (*R*) correspond with the Malcostraca (*O* and *P*). It is evident that Sollaud's 'l'aire blastoporique primaire' of the Crustacea corresponds with the ventral wall of the yolk sac in the Onychophora, and that the germinal bands in early crustacean stages cannot be considered as 'manifestement comparables aux bords libres, périblastoporiques'. Since Sollaud's claim that immigration of endoderm proceeds to a small extent from 'l'aire blastoporique primaire' has not been confirmed by either Manton or Nair on other Malacostraca, and as Sollaud's figures indicate that his technique is not as good as can be obtained, there appears to be no reason for considering the blastopore in the Malacostraca to be represented by anything other than the 'blastoporal area' of Manton and Nair or 'l'aire blastoporique secondaire' of Sollaud.

The second likeness between Annelida and Onychophora quoted on p. 541 needs amendment. Concerning the origin of mesoderm: the proliferating area may lie behind an open blastopore as in *Astacus*, and then corresponds in position to that of the blastomere 4*d* in a polychaete, but more usually the proliferating area in Onychophora and Arthropoda either succeeds the blastopore in time or replaces it, and hence is termed a blastoporal area. When no blastopore is present or when it is obliterated early, mesoderm arises from the blastoporal area. Concerning the forward growth of mesodermal bands in the Onychophora: their development is simpler than it is in most annelids as well as arthropods, see next section.

Primary and secondary metamerism in segmented animals, and the primitive character of the Onychophora

Much has been written recently concerning primary and secondary segmentation (Iwanoff 1928, 1933 and 1944; Snodgrass 1938), which Iwanoff (1944) claims to have the significance of a new principle of morphology. A review of this theory seems called for, as the facts appear to be open to a somewhat different interpretation.

* Neither Nair nor Manton has observed any extra blastoporal immigration in the Malacostraca, other than that of the pre-antennulary mesoderm which comes from the germinal bands.

Iwanoff has drawn attention to the 'primary larval segments' in Annelida, Arthropoda and Enteropneusta, etc., arising in a manner unlike that of the post-larval or 'secondary segments' which comprise most of the adult body. Snodgrass follows this up by attaching a phylogenetic significance to these ontogenetic phenomena, and interprets them as indicating the manner in which metamerism may have arisen in evolving segmented animals (see below).

The *primary segments* of polychaetes appear almost simultaneously in the trochosphere. Their mesoderm arises from the mesodermal bands descendent from the blastomere 4*d*. The segmentation of these somites appears to be secondarily imposed upon them by metamerically arranged structures, such as chaetal sacs and mesenchyme muscles, and by ectodermal segmentation (Meyer 1901; Iwanoff 1928). These mesodermal somites are first solid and become hollow later. The primary segments are few in number, three are present in *Eupomatus* and other serpulids, various numbers in different spionids, thirteen in *Chaetopterus*, etc. These anterior larval segments may show structural differences from the post-larval or secondary segments in their blood system, their nephridia (which may be absent in the former), and in the absence of germ cells, and the larval segments may be ill-formed in the adult. Regeneration from the larval segments results in re-formation of these segments only and not of the secondary segments, which normally arise from a growth zone. Regeneration from the secondary segments, however, can replace the whole body.

The *secondary segments* in polychaetes usually arise from a pre-anal ectodermal growth zone. They often appear after a pause in development associated with the dedifferentiation of larval tissues. No undifferentiated cells are present in the larva at this stage, and the whole of the descendants of 4*d* have become differentiated larval structures. From the growth zone new ectoderm is formed, and new mesoderm arises by immigration of numerous ectodermal cells (from the dorsal part only of the growth zone in *Eupomatus*). This mesoderm is entirely distinct from the mesodermal bands descendent from 4*d*.

A comparable distinction between primary and secondary segments occurs in other groups. In the Oligochaeta, Iwanoff has pointed out that the head segments (comparable with the larval or primary segments) differ structurally from the rest in their vascular system, in the absence of chloragogen cells, of nephridia, and of gonads, and of septa also in the Naididae. They number five in the Tubificidae, seven in the Lumbricidae, etc. Iwanoff has shown that part of the mesoderm of these segments is 'trochophoral' or primary mesoderm, but that the rest of the mesoderm of these segments arises from the somatopleur of the metatrochophoral or secondary segments, which spreads forwards and encloses the larval mesoderm. Different regeneration capacities of the primary and secondary segments can be found which resemble those of polychaetes, but exceptions also occur, as in *Criodrilus*, where the primary segments are capable of regenerating not only larval segments, but also a series of post-larval segments with genital organs (Janda 1912).

In the oligochaetes, in the polychaetes *Arenicola* and *Aricia*, and in *Polygordius* all the mesoderm, both primary and secondary, comes from 4*d*. Iwanoff therefore suggests that the essential difference between primary (trochophoral) and secondary (post-trochophoral) mesoderm lies not in its origin but in the character of the metamerism, the former being dependent upon other organs for its segmentation. In *Arenicola* the growth of the somites from the mesoderm of the pre-anal growth zone has been described by Lillie (1905).

A distinction between primary and secondary segments is not at all clear, but Child's work (1917) on metabolic gradients in *Arenicola* has been interpreted by Iwanoff as providing physiological evidence of the presence of at least three primary segments. In *Polygordius*, where all the mesoderm comes from 4*d* and all the somites can form germ cells, Iwanoff draws the rather unhappy conclusion that all segments must here be secondary, the larval tissues being limited to the prostomium. A different interpretation is suggested below (p. 550).

In many Arthropoda, excepting the Onychophora, a difference between primary and secondary mesoderm can also be found. In *Limulus*, according to Iwanoff (1933), one particular blastomere gives rise to the primary mesoderm supplying the four segments from the cheliceral to that of the second walking legs; this mesoderm either has no clear coelomic cavities, or the cavities are of short duration, and the mesoderm soon breaks up into separate groups of cells forming the heart, pericardium, etc. The secondary mesoderm forms the sixth somite backwards, and arises from a blastoporal area (primitive streak), regular mesodermal bands and coelomic sacs with single-layered walls being formed. The fifth segment obtains its mesoderm from both sources.

In the malacostracan Crustacea the 'naupliar mesoderm' supplying the antennulary, antennal and mandibular segments (Manton 1928, 1934; Nair 1939, 1941) arises from mesodermal bands which segment almost simultaneously but do not become hollow,* and the secondary mesoderm supplying the maxillulary segment backwards is teloblastic in origin, arising from a limited number (8 or 10, etc.) of large teloblasts. No teloblasts have so far been seen in other crustacean groups.

In the symphylan *Hanseniella*, which Tiegs (1940, 1945) has so admirably shown to possess many features of the ancestral insectan stem, a distinction between primary and secondary mesoderm can perhaps be made. The seven embryonic somites arise from mesoderm which separates from a large area of the germinal band, and could presumably be called primary. The succeeding five segments are added during larval life in a complex manner from the 'pre-anal' and 'anal pieces', and their somites are formed from the anal somite, the undifferentiated mesodermal band and the penultimate somite, details of which are fully given by Tiegs. These secondary somites (if the term is here justified), once they are formed, develop further in almost but not quite the same manner as the primary somites, giving rise to a similar set of organs excepting a splanchnic component which is not formed by them.

In the Enteropneusta Iwanoff points out that the first three pairs of coelomic sacs appear almost simultaneously in the larva, and that the post-larval segmentation is a consequence of incomplete division of the third segment, so presenting a parallel to the primary and secondary segmentation in the phyla already mentioned.

Since primary and secondary metamerism can be found in chordates and annelids, Iwanoff (1944) suggests that it has a profound morphological significance. Snodgrass(1938) pushes the matter further, giving it unqualified phylogenetic significance, and suggests that the ancestral primarily segmented animal comprising a few segments extended its body by secondary segmentation 'as a means of amplifying the reproductive function', so resulting

* The antennal gland end sac arising later probably represents a coelomic space of the antennal somite (see Manton 1928).

eventually in modern Annelida, Onychophora and Arthropoda, a conclusion against which there is considerable evidence, as will briefly be shown.

It has long been recognized that larval features do not recapitulate those of a series of ancestral adult stages, but can only claim to recapitulate in part the ancestral ontogeny (Garstang 1922; de Beer 1930; Needham 1930, etc.). Thus a larva with primary segments only does not represent an adult ancestor with few segments, but only the larva of an ancestor, and the formation of a post-trochophore by secondary metamerism does not necessarily represent the phylogenetic origin of a many-segmented animal from one with few segments. These ontogenetic stages can only represent phases in the ontogeny of the ancestral line. Many larval features have no phylogenetic significance and may be interpreted as specializations correlated with larval habits of life. Thus processes appertaining primarily to larval developments need very cautious interpretation. There appear to be two main approaches to an understanding of primary and secondary metamerism which do not involve the highly doubtful interpretation of phylogenetic recapitulation.

Iwanoff (1928) suggests that the two methods of growth of the mesoderm in many polychaetes may be due to a need for more efficient functioning of a pelagic larva. The latter is clearly of paramount importance, and the need for early functioning with maximum efficiency of a small part of the body is present in all such animals, no matter how remotely related may be their phyla. This surely is the explanation of the parallel occurrence of 'primary segments' in the Enteropneusta and Polychaeta, and of the precocious development of ectodermal structures, such as muscles, chaetal sacs, ciliated bands, etc., all of which enable the larva to meet the struggle for existence with the greatest efficiency at the earliest possible moment, and long before the whole body has been laid down.

Secondly, during development a two-layered condition usually precedes a three-layered state. The ectoderm, which is present when mesoderm formation starts, may be sufficient to supply some but not all segments. Thus the first-formed mesoderm passes under pre-existing ectoderm to give the anterior or primary segments. The posterior segments, however, may need new ectoderm as well as mesoderm for their initiation, and both of these arise together from a growth zone. This is seen particularly clearly in the Malacostraca where the junction of naupliar (primary) and teloblastic (secondary) ectoderm can be traced exactly. The blastoderm present at the end of segmentation supplies the ectoderm of the naupliar segments only, and secondary (teloblastic) ectoderm and mesoderm arise simultaneously from either a peri- or a pre-blastoporal growth zone (Manton 1928, 1934; Nair 1939, 1941; and figure 6*P* and *Q*). Indeed, it would be surprising to find many examples of segmented invertebrates whose development was uninfluenced either by the early possession of ectoderm sufficient for some but not all segments, or by the presence of a larva with precocious functional organs. Thus it is suggested that the phenomenon of primary and secondary metamerism would be expected to appear for the above reasons, and not because a short-bodied ancestor had increased its length by secondary segmentation.

The presence or absence of germ cells has been used as a criterion of primary and secondary segmentation by Iwanoff for the Polychaeta, germ cells being absent from the primary segments. It is difficult to place much significance on the exact location of the germ cells. They are differentiated very early in some groups and later become associated with coelomic sacs (see p. 533), and their absence in the primary segments may be associated

with the specialized larval functions of these segments which do not include that of reproduction, and with the reduced state of these segments in many adults. The interpretation of *Polygordius* as consisting entirely of secondary segments (Iwanoff 1928), apart from the prostomium, seems questionable. If the presence of primary and secondary segmentation is divested of a phylogenetic interpretation concerning the origin of many-segmented animals, then surely *Polygordius*, with all its segments arising in a similar manner, and with all the mesoderm descendant from the cell 4*d*, is more reasonably interpreted as showing a simple basic method of development in which there is no distinction of primary and secondary segments. This condition is possibly preserved to-day because the prostomium of *Polygordius* is itself sufficiently large and specialized to serve the animal's needs during planktonic life, so that no anterior segments are modified for larval functions.

Turning now to animals with no early larvae or with yolky eggs. If primary and secondary metamerism is here evident, it may be regarded as a recapitulation by the embryo of the ontogeny of a larva-bearing ancestor which possessed this feature. The conditions in many Oligochaeta and Arthropoda seem to be reasonably so interpreted. The reduced state of the primary segments of the Oligochaeta may be correlated with the suppression of early larvae, and may have led to the lessened extent of the primary mesoderm. However, in the Arthropoda with no early larvae, a differentiation of primary and secondary mesoderm may be very clear, as in *Limulus* and the Malacostraca. The ectoderm is also sharply demarcated into primary and secondary regions corresponding to the mesoderm in the Malacostraca, but this appears not to be the case in most Arthropoda. Where yolk becomes voluminous, there may be enough ectoderm surrounding it for the elaboration of many more segments than the original primary ones, so that primary and secondary metamerism may become evident mainly in the mesoderm, as in *Limulus* and *Hanseniella*.

In the Onychophora it has been shown that there is extraordinary uniformity in the development of segmentation throughout the whole body. The procedure is far simpler than that seen in most existing Annelida and Arthropoda (pp. 521 and 533), and there is no trace of primary and secondary metamerism. The whole of each mesodermal band arises similarly from the blastoporal area, and somites are separated progressively from before backwards. Only the first few somites may develop small cavities before they are cut off from the band, and all others are separated in a solid state and become hollow later (p. 522); this difference is irregular and does not seem to have any particular significance, certainly it is not indicative of heteronomy. The subsequent development of the somites is extraordinarily similar throughout the body, and even the antennal pair forms rudimentary and evanescent segmental organs.

What is the meaning of this extreme simplicity? There appear to be two plausible hypotheses, the simplicity may be either primitive or secondary. If the simplicity is *primitive*, then the Onychophora show a much simpler ontogeny than do the vast majority not only of existing Arthropoda but of Annelida also. They show a simple condition which must have preceded the development of primary and secondary metamerism. This simplicity might be correlated with the presence of a simple larva (or early developmental stage) by the marine ancestor in which either no specializations existed for larval life, or in which such specializations were limited to the prostomium, and thus would not call forth heteronomy.

Alternatively, the present simplicity may be *secondary*, and a result of the long embryonic development and viviparous habit. This habit has clearly replaced an oviparous yolky one (p. 535), and the latter must in its turn have replaced less yolky free living ontogenetic stages of a marine ancestor. With the attainment of either a prolonged oviparous or a viviparous state and the loss of free developmental stages, any need for heteronomy would disappear, and all traces of the process might be supposed to vanish. The large size of the yolk sac might have facilitated an obliteration of heteronomy, as in species such as *P. moseleyi* an abundance of ectoderm sufficient for more than half the segments of the embryo is present when mesoderm formation starts, and no new ectoderm is needed for a time.* However, the mesoderm shows the same uniformity in species both with large and with almost obliterated yolk sacs.

The conditions in the yolky Arthropoda render a secondary loss of heteronomy in the Onychophora improbable. Many arthropodan groups show heteronomy of the mesoderm although there appears to be no functional need for it, as early larvae have been suppressed. If heteronomy in the Arthropoda is due primarily to the amount of ectoderm present at the onset of mesoderm formation (see above), then the same should hold for the Onychophora, and a persistence of heteronomy in the Arthropoda and its obliteration in the Onychophora would remain unexplained. The general occurrence of heteronomy at least of the mesoderm in Arthropoda would thus appear to be correlated with the possession at some phylogenetic stage of specialized larvae in which the primary and secondary metamerism arose for the reason suggested on p. 549. Such specialized larvae must have been absent from the ancestors of the Onychophora. Further, the anterior somites of the Onychophora show very generalized form and functions, and it is difficult to conceive of this feature being either retained or reacquired if heteronomy had intervened.

Thus the evidence is in favour of the absence of distinction between primary and secondary segmentation in the Onychophora being primitive. This primitiveness is not characteristic of most members of the Annelida and Arthropoda, but a comparable condition is perhaps to be found in *Polygordius* and a few polychaetes. The Onychophora thus appear to have diverged from the annelidan-arthropodan line at a very remote period, before the modern annelids had acquired either a characteristic trochosphere larva or their heteronomy. Such a conception of the great antiquity and isolation of the Onychophora is not at variance with other data.

The above review thus does not support the suggestion by Iwanoff (1944) that the type of heteronomy under discussion 'is typical for primitive forms of all metamerical animals'. Neither does it support the view of Snodgrass (1938) that a short-bodied ancestor with a few primary segments 'acquired a lengthened body by the addition of secondary genital somites proliferated from a sub-terminal zone of growth' to form the progenitor of the Annelida, Onychophora and Arthropoda. It has been shown that this heteronomy may be expected to arise in association with specialized larvae which function early. Primitively this heteronomy must be absent, as is seen in a few annelids and in the Onychophora to-day, and secondarily it may be partially obliterated as in the Oligochaeta and many Arthropoda. No direct evidence is available as to how the annelidan-onychophoran-arthropodan pro-

* Interstitial growth of the ectoderm occurs all along the germinal bands, leading to increase in size, the formation of limb buds, etc.

genitor acquired its many segments from an unsegmented condition, but it is probable that all segments arose in a similar manner and that the free living ontogenetic stages of this progenitor were unspecialized.

Formation of mesodermal bands in Arthropoda

The most primitive method of formation of mesodermal bands in annelids and arthropods seems to be that shown by those annelids and archiannelids and also Mollusca in which the derivatives of the blastomere 4*d* form the whole of the band, as in *Arenicola* and *Polygordius*. In the Onychophora and Arthropoda cell lineages usually cannot be traced but in some cases the mesoderm has been seen to arise from a few cells, two blastomeres in the Malacostracan *Euphausia*, five in *Lepas*, etc. More usually a many-celled origin of the mesoderm occurs, and then the more primitive condition appears to be one where the mesoderm arises either from a small blastoporal area or primitive streak (Onychophora, Malacostraca, etc.), or from a zone behind an open blastopore (*Astacus*). Specialization in the annelids may lead to a secondary formation of mesoderm from the ectoderm of a pre-anal growth zone supplying the secondary segments, a process recalling the ability of many adults to form both ectoderm and mesoderm at regeneration from the ectodermal layer. Specialization in the Arthropoda may also lead to a secondary formation of the posterior mesoderm, an extreme example being the mesoderm of the Malacostraca; this method of mesoderm formation, often by eight teloblasts which are not present in the earliest stages, cannot be regarded as a primitive feature. There is no reason to doubt that the head band mesoderm of the Malacostraca supplying the naupliar segments represents part of the primitive mesodermal band of the ancestral arthropod or annelid, which is seen to-day in the Onychophora. Another arthropodan specialization seems to lie in the expansion of the area from which primary mesoderm is formed; such an expansion is seen in some Arachnida, in the Symphyla and Insecta, and ultimately the mesoderm may arise from a very large part of the germinal disk, an elongated zone of immigration or invagination extending along the whole length of the disk.

Origin of metamerism

Snodgrass (1938), in his review of the mesoderm and of the initiation of metamerism, supports the view that 'metamerism had its origin as an adaptation to more effective body movement', an opinion which must meet with general approval. In seeking a phylogenetic origin of metamerism, however, he places a phylogenetic interpretation on polychaete ontogeny which is unjustifiable (see above p. 548). He suggests that metamerism arose phylogenetically in the ectoderm from primary ectodermal longitudinal muscles which were attached at consecutive rings of the body wall, followed by ingrowth of fibres between the myotomes. He considers that 'segmentation is a feature superimposed upon the mesoderm in the Annelida as a result of body metamerism', as seen in the ontogeny of the primary segments in polychaetes where the continuous mesodermal bands may be segmented by the ingrowth of chaetal sacs, the penetration of mesenchymatous muscles, or by the formation of blood lacunae, etc. He states that 'the definitive musculature of modern annelids...is a composite of fibres derived from the larval ectoderm and of fibres formed

from the coelomic mesoblast, but in the Onychophora and the Arthropoda the entire musculature appears to be now a coelomic product'. He claims that mesodermal 'coelomic sacs do not determine metamerism', but that their cavities are secondary phenomena appearing subsequent to segmentation, probably in association with nephric functions.

The above views will not survive much criticism. Reference to the fallacy of placing a phylogenetic interpretation on features in a trochosphere larva, where a premium is put on the early functioning of some organs, has already been made (p. 549). A precocious utilization of the ectoderm for organ formation before the mesoderm has become established is to be expected. Setting trochosphere larvae aside, what evidence is there of metamerism being superimposed on the mesoderm by the ectoderm, or, on the other hand, of its originating in the mesoderm?

An independent ontogenetic segmentation of the mesoderm is very striking in many Arthropoda. In the Onychophora the initiation of metamerism undoubtedly lies in the mesoderm, which segments *before* there is any trace of this process in the ectoderm (pp. 521, 523 and 554). The present work does not uphold the statement that in 'the Onychophora... as in the Annelids, the primary solid mesoderm bands are first segmented corresponding with the body somites and then excavated by coelomic cavities'. The contrary has been shown to be the case (pp. 519 and 521), and is illustrated by the whole views and reconstructions on plates 31, 32, 33, 34, 37 and 38, where mesodermal segmentation precedes all trace of ectodermal segmentation. Note, for example, figures 11 and 12 where 10 and 20 mesodermal somites are respectively present, yet only 2 and 6 segments respectively are shown by the ectoderm, also figure 32 where one mesodermal somite is present and no ectodermal differentiation, and figure 92 where 10 mesodermal somites are present, but only 4 segments are visible in the ectoderm. When segmentation of the mesoderm is established the ectoderm follows the contours of the blocks of internal tissues, and shows bulges and furrows corresponding to their metamerism. Thereafter organogeny of both layers proceeds simultaneously. The present work supports Sedgwick's view (1884) that 'the mesoblast is the first part of the body to show segmentation. The rest of the segmentation is moulded on the segmentation of the mesoblast.'

The Crustacea, as well as the Onychophora, show clear examples of metamerism arising first in the mesoderm. In the longitudinal section of the trunk region of a metanauplius of *Chirocephalus* Cannon (1926) shows a segmented mesoderm lying within an undifferentiated ectoderm, and Weisz (1947) writes of the mesoderm in the developing *Artemia salina* 'it initiates segmental development'. Of the symphylan *Hanseniella* Tiegs (1940) writes 'in the mesoderm segmentation begins a little earlier than in the germ band itself', and Iwanoff's text-figure 18 (1933), illustrating the secondary segments of *Limulus*, shows the same thing. The Annelida also provide equally clear examples of mesodermal segmentation preceding that of the ectoderm (see Lillie's figure 33, plate 24 (1905) of *Arenicola*, etc.).*

* Experimental work on vertebrate embryos has shown that the 'segmentation of spinal ganglia and associated nerves is subservient to mesodermal metamerism' (Detweiler 1934); where supernumerary somites are implanted there follows a corresponding increase in the number of spinal ganglia and peripheral nerves (Detweiler 1938). Thus vertebrates also show examples of the mesoderm initiating the metamerism of other structures.

In the malacostracan trunk region where ectodermal and mesodermal teleoblasts are operative, the initiation of metamerism cannot be said to lie in either layer, as orderly ectodermal and mesodermal rudiments of each segment are laid down simultaneously.

These facts cannot be explained by Snodgrass's view that the ectoderm in Arthropoda no longer carries out its primitive muscle-forming functions and thus leaves the mesoderm to segment, apparently autonomously, as a secondary phenomenon. The ectoderm of certain Crustacea has been shown to form many structures such as endoskeleton, fibrils, unstriated and striated muscle, either remaining in contact with the adult ectoderm or becoming far removed from it (Cannon 1926, 1931 and 1940; Manton 1928, 1934).* The ectodermal derivatives vary greatly from one animal to another; for example, muscles of similar function and position may arise from mesoderm in some animals and from ectoderm in others. Yet despite the great potentialities of arthropodan ectoderm, this layer does not initiate mesodermal segmentation.

The following statement 'that coelomic sacs do not determine metamerism is shown by the formation of paired coelomic cavities in pre-oral cephalic mesoderm of the Onychophora and Arthropoda, in which there is no external segmentation' (Snodgrass 1938) is also unacceptable. In some species of Onychophora the first pair of somites may be differentiated in a post-oral position (figure 84, plate 38), and later, when they have shifted to a pre-oral position and have enlarged, ectodermal segmentation becomes apparent. The antennal segment in the Onychophora is as clearly demarcated from the next as are any other consecutive segments of the body (see plates 31, 33 and 38) (the antennal somites are here considered to be serially homologous with those following them; see next section). In the Arthropoda with yolky embryos intersegmental furrows are frequently feebly developed or absent in early stages and may become apparent later in association with the formation of segmental limb bulges, ventral organs, intersegmental components of the endoskeleton, etc. Thus segments rudimentary in the adult, such as the pre-antennulary segment in Crustacea, show no intersegmental ectodermal furrows delimiting their extents, but the more posterior limb-bearing segments may be equally devoid of such boundaries in corresponding early stages (Manton 1928, 1934). Thus the absence of marked external segmentation is no indication that metamerism is also absent.

Thus it seems reasonable to regard metameric segmentation as primarily associated with the mesoderm. This segmentation may have originated more than once, and consecutive attachments of mesodermal muscles may have been a causal factor. A semblance of segmentation of the dorso-ventral mesodermal muscles is already seen at the anterior end of a land planarian (Pantin, unpublished observation†). Moreover, powerful muscles in animals do not tend to lie in a superficial position; they take on a deeper location, within

* A claim to the contrary for *Neomysis vulgaris* has been made by Needham (1937), to which Cannon has replied (1940, p. 198). The methods employed by Needham were not such as could demonstrate the presence or absence of ectodermal muscles, tendons, etc., with certainty, and even should his claims be substantiated for *Neomysis* by work carried out with better technique, it would have little bearing on the presence of ectodermal muscles, etc., occurring in other Crustacea. The development of a third mysid, *Mesopodopsis orientalis*, has been studied by Nair (1939); he has not followed the development of the muscles found to be ectodermal in *Hemimysis*, but he describes the liver to arise from the mesoderm, just as occurs in *Hemimysis*, while Needham finds an endodermal liver rudiment in *Neomysis*.

† The planarian is identical with *Rhynchodemus* (*-Geodesmus*) *bilineatus* (Metchnikoff).

the mesogloea in the tentacles of large medusae, deeply seated in the mesenteries of Actiniaria, and in the parenchyma in planarians. A metamerism of mesodermal musculature is more likely to be a predominant factor in the evolution of a many-segmented animal than is an addition of 'secondary segments' for enlarging the genital functions.

Composition of the head in Onychophora

The criteria of a segment have been enumerated (Goodrich 1897; Tiegs 1940, etc.) and do not need repetition or amplification. No reference will be made here to the evidence provided by the nervous system concerning the segmentation of the head, as no new data relating to the brain are here presented. The nervous system develops later than the stages of the Onychophora here recorded (see however p. 561).

It has been shown that the initiation of metamerism in the unsegmented onychophoran embryo lies in the mesoderm; it does not lie in the ectoderm. The basic uniformity in the formation of the paired mesodermal somites in *Peripatopsis* has been described and discussed (pp. 521 and 546). It is clear that the antennal somite arises in exactly the same manner as do the succeeding somites, and its early development is the same. Like the majority of somites it develops a coelomoduct, but this structure disappears later. The antennal somite is undeniably post-oral in origin (figures 84, 86, 114, 116 and 117, plates 38 and 41, and figure 7), and it migrates forwards in the same manner as do the succeeding somites, but unlike them it is the only one to become completely pre-oral. The antennal segment is demarcated externally from the succeeding segments in early stages just as the trunk segments are from one another, and all segments have their ventral organs which contribute to the formation of the nervous system. The unsegmented parts of the embryo comprise those regions which do not contain mesodermal somites; in early stages they lie anterior and posterior to the somites, and above and below them in the form of the dorsal and ventral walls of the yolk sac (see *P. moseleyi*, figures 33 and 34, plate 33). These unsegmented regions are in part progressively incorporated into the expanding segments, and partly obliterated by absorption (see the plates). The extent of an unsegmented anterior acron (prostomium) and posterior telson in the adult cannot be determined, as the unsegmented embryonic regions merge insensibly into the segmental parts as the yolk-sac walls become absorbed. There appears to be no sound evidence from either the adult structure or from the embryology of the Onychophora against the view that the antennal segment is serially homologous with the succeeding segments. (An alternative interpretation has been elaborated by Snodgrass (1938); see p. 558.)

There is at present no acceptable evidence of the existence of a pre-antennulary segment in the Onychophora. The two recorded cases of mesoderm lying in front of the antennal somites, a single embryo of *Eoperipatus weldoni* (Evans 1901) and the embryo of *Peripatopsis* described above on p. 525, are probably exceptional abnormalities of no theoretical importance. Out of the hundreds of embryos covering five species of *Peripatopsis* examined for the present work, only one embryo has been seen with any trace of mesoderm in a pre-antennal position, and this one is asymmetrical. Numerous embryos have been found, some of which are described above (pp. 507, 523 and 525) which show somites in abnormal sizes, positions and numbers. As these animals are subject to so much variation, little if any significance can be attached to either of these embryos. Kennel (1886) records the transitory

appearance of a pair of small prominences in front of the embryonic antennae of *Epiperipatus edwardsii*. Until further details are known little can be deduced from this observation. Thus, considering the Onychophora alone, the available evidence indicates that the antennal segment is the first segment of the body; it is the only segment which has become pre-oral, and to it are fused the remains of the anterior unsegmented acronal part of the embryo.*

Morphology and phylogeny of the head in Onychophora and Arthropoda

It is not proposed to give a complete review of this vexed subject. Certain principles will be considered, and the bearing of the present work on some recent theories will be given.

Much has been written concerning the segmentation of the head and body in Arthropoda based on the numbers and positions of embryonic mesodermal somites. Much unnecessary confusion has resulted from a lack of appreciation of data drawn from all arthropodan groups, and from a disregard of the following three points:

(1) The boundaries of an embryonic somite in most parts of the body are obvious, but where two coelomic sacs are found either connected together or even separate from each other, this fact alone forms no conclusive evidence for the view that the two sacs represent somites of two adjacent segments. The possible fallacy of considering the 'labral' and 'pre-antennal' coelomic sacs seen in a certain stage of the stick insect *Carausius*, as separate segmental entities was pointed out in 1928. A single somite normally divides into two parts in this region, the precheliceral of the spider (Kishinouye 1893), the pre-antennulary in the Malacostraca (Manton and Nair), and the antennal in *Eoperipatus* (Evans 1901), but this functional division is no evidence that two segments are involved, a view with which Tiegs (1940) is in agreement. Where organ formation from somite walls is precocious, as in many insects, it would be a small step for two functional parts of a somite to separate partially or completely at an early stage, even before any coelomic space had appeared. But this would provide no evidence for the existence of two originally separate segments.

(2) There are examples of united somites which do represent more than one segment, but the early development of such structures may show their dual nature, as, for example, the fused maxillary and first thoracic coelomic sacs in *Hemimysis*.

(3) Although most coelomic spaces arise each from a single lacuna within the somite mesoderm, which then gradually enlarges, some coelomic spaces may arise from several lacunae in a mesodermal band when the embryo is relatively large, as do the anterior somites in *P. moseleyi* and *P. capensis* and in the species shown in figure 5*a* to *d* (p. 524). The existence of multiple initial spaces is no evidence that each of these spaces represents a segmental unit.

In an interpretation of any one animal the facts of development of that animal must be adequately considered with appreciation of the above points, and comparisons must be made with other Arthropoda. A disregard of these principles has led to faulty arguments and confusion in the literature.

Lastly, there is a further possible cause of variation in segment numbers in the tagmata of arthropods, which is not usually operative in head formation, but which may possibly

* This conclusion is at variance with that of Holmgren (1916) and Hanstrom (1928), who on neurological evidence alone consider the jaw segment of the Onychophora to be the first segment of the body see (see pp. 558 and 561).

occur in some cases. It has been shown (de Beer 1930, etc.) that occasionally the mesoderm destined for a given zone can segment into an abnormal number of somites on one side of the body. If this occurred on both sides an extra segment might appear. It is possible that the extra-legged condition of some Pycnogonida may have arisen in this manner. Calman & Gordon (1933) drew attention to the 'disturbance of metameric pattern affecting the number of cephalic metameres, similar to the disturbance which we believe to have affected the trunk segments in the decapodous genera', and Hedgpeth (1947) has further discussed the polymerous condition. It is possible that the mesoderm of the head of some insect or other arthropod might segment into more than the normal number for the group, as occurs in some Pycnogonida. There is no indisputable evidence of any insect having more than six head segments (see Tiegs 1940, and below), but if a fourth procephalic segment should be shown to be present undeniably in any one insect, as claimed by Roonwal (1937, 1938 for *Locusta*), it might be accounted for as suggested above, and the segmentation of such an insect would not be rendered incomparable with other mandibulate arthropods.

The theory of arthropodan head segmentation elaborated by Snodgrass (1938)

TABLE 2. TABLE SHOWING THE RELATIONSHIP BETWEEN THE SEGMENTAL INTERPRETATION OF THE ARTHROPOD HEAD SUPPORTED BY GOODRICH (1897) AND OTHERS, AND THE ALTERNATIVE INTERPRETATION PUT FORWARD BY HOLMGREN AND HANSTRÖM AND ELABORATED BY SNODGRASS (1938).

	Annelida	Onychophora	Arachnida	Crustacea	Myriapoda
primitive pre-oral region	prostomium	unsegmented acron			
segment 1	*M* *peristomium	antennae	precheliceral	pre-antennal	pre-antennal
		M			
„ 2		*jaws	*chelicerae	antennules	antennae
			M		
„ 3		slime papillae	pedipalpi	*antennae	*premandibular
				M	*M*
„ 4		legs	legs	mandibles	mandibles
„ 5		legs	legs	maxillules	maxillules
„ 6		legs	legs	maxillae	maxillae

Similar horizontal levels show homologous segments, and their appendages, behind the unsegmented acron on the former theory, and the position of the mouth is indicated by *M*. The line surrounds the regions suggested by Snodgrass and others to be the unsegmented acron, and the asterisks mark the segments suggested to be the first segment of the body in each group on the latter theory.

The classical interpretation of the adult arthropod head is that it consists of an unsegmented acron, comparable with the annelid prostomium, with which are fused a variable number of trunk segments, this number being constant in each group (Goodrich 1897, etc.). The mouth, originally in front of the first segment embryologically, and presumably phylogenetically also, is seen to shift during development relative to the first few segments so that one or more become pre-oral. Lankester (1873) first suggested that the mouth of arthropods must have shifted backwards, and this movement has clearly been seen in the Onychophora (figure 7), in the crustaceans *Hemimysis* and *Nebalia* and in *Pauropus* (see p. 559). In the Crustacea, Myriapoda and Insecta the head appears to consist of six segments. Six pairs of hollow coelomic sacs only have been found in insects by Eastham (1930), Mellanby (1936),

Roonwal (1936), etc., and there is as yet no good evidence that more than six somites are represented in the head of any insect (Tiegs 1940).* Three of these have become pre-oral. In the Onychophora only the antennal segment shifts to this position. On this interpretation the antennal segment in *Peripatus* corresponds with the precheliceral of the Arachnida, the pre-antennulary of the Crustacea and the pre-antennal of the Myriapoda (see table 2). The present work supports this theory as applied to the Onychophora and to the mandibulate groups, but no attempt is here made to reconcile the opposing interpretations of the head in the Arachnida (see below). Tiegs (1940) has discussed the segmentation of the head in Myriapoda† and Insecta, and as the present work is in complete agreement with this discussion, the matters there raised will not be considered further here.

A different interpretation of the arthropod head has been put forward by Holmgren (1916) and Hanström (1928) based upon the comparative morphology of the adult nervous system alone, and this view has been further elaborated by Snodgrass (1938). The primitively unsegmented acron is supposed to have become progressively developed in the several arthropod groups. It is said to bear the eyes and labrum, the antennules and antennae in the Crustacea, and the antennae in the Insecta, Myriapoda and Onychophora. In accordance with the neurological findings of Holmgren and Hanström, the first segment of the body, or tritocerebral segment, is considered to be the jaw segment of the Onychophora (behind the mouth), the cheliceral segment in the Arachnida, the antennal segment in Crustacea, and the premandibular segment in Insecta (all in front of the mouth in the adult); these segments are marked by an asterisk in table 2.

The opinions of authors supporting the 'tritocerebral nature of the chelicera' in the Arachnida have been summarized by Stormer (1944) and others. The view that the cheliceral segment is the first body segment is based upon neurological foundations without reference to the mesoderm. Yet the existence of a precheliceral segment 'est absolument hors de doute' (Dawydoff 1928). This segment usually bears no limbs, but its pair of mesodermal somites have been described and figured in the spiders *Agelena* and *Dolomedes* by Kishinouye (1893) and Pappenheim (1903) respectively, and Heymons (1905) has given brief details of the somites in *Galeodes*. Workers on the embryology of other Arachnida have not found separate precheliceral somites. Further information concerning the cephalic mesoderm of the Arachnida is clearly desirable, as the significance of mesodermal segmentation appears to have been underestimated in the group. In both vertebrates and invertebrates metamerism of the mesoderm has been shown to determine that of the ectoderm, nerve ganglia, nerves, etc. (see pp. 520 and 553), and evidence of head segmentation provided by the mesoderm should not be ignored in any consideration of the Arachnida.

The evidence presented by Snodgrass (1938) for the above-mentioned interpretation of the arthropod head will now be considered. He elaborates the suggestion that coelomic spaces can occur secondarily in the prostomium. In the polychaetes the first pair of trunk somites may invade the prostomium, and in spionids a separate pair of coelomic sacs is

* There is no need to discuss the evidence for separate labral and pre-antennal segments in insects (Weismann 1926; Roonwal 1937), as this has already been done by Tiegs (1940) and Manton (1928).

† Concerning the phylogeny of the Symphyla, Tiegs (1940) concludes that they are 'a group of very primitive Myriapods, whose development discloses unexpected evidence of affinity with *Peripatus*, while their more immediate relationships lie with the diplopods on the one hand, with the insects on the other, but not with the chilopods.'

associated with the palpi (Binard & Jeener 1928). He suggests that a similar explanation may account for antennal and pre-antennal coelomic spaces in his extended arthropodan 'acron', and that the formation of cavities in the cephalic mesoderm is a secondary accompaniment of advancing organization of the 'prostomial lobe' and is not indicative of metameric segmentation. This view appears to have little to commend it. In the Arthropoda parts of somites may become specialized and separate, but this is not to be confused with the appearance of a segmental series of true somites (see above, p. 556). If this view is applied to the Onychophora it raises profound difficulties. If the antennal segment is no segment but only part of an unsegmented acron, how are the striking similarities (shown above) between the antennal somites and those behind it to be explained? If the characteristics of the antennal segment are not indicative of its true segmental nature, there is little justification for applying that term to any other segment of the body. When this theory is applied to other Arthropoda it again raises difficulties. It does not explain the amazing uniformity of the plan of mesodermal segmentation found in the heads of the mandibulate groups in spite of the difference in the numbers of adult head appendages. Snodgrass's statement that coelomic sacs in his extended 'acron' are best developed in the higher arthropods is not borne out by the facts, unless he means that two somites occur in his 'unsegmented acron' in the mandibulate arthropods while only one occurs in *Peripatus*. In the Crustacea, for example, a hollow antennulary somite has not yet been seen, and the antennal somite is solid except for the cavity of the antennal gland. The cavity of the antennal somite in *Peripatus* is relatively much larger than any in the Crustacea. Tiegs (1940) correctly remarks concerning the Symphyla and Insecta that 'in all head segments there is a tendency for coelomic sacs to disappear'.

Snodgrass submits a summary of the evidence for his views under eight headings (1938, p. 94); the more important of these will shortly be considered.

(1) Snodgrass states that 'there is never any external division of the acronal region (his extended acron) into segmental areas', and he refers to the work of Sollaud (1923) showing the first intersegmental furrow in a palaemonid prawn to lie behind the antennulary somite. It is true that no furrow has been found to lie between the embryonic pre-antennulary segment and the antennulary segment in Crustacea, or between the pre-antennal and antennal segments in the symphylan *Hanseniella*, but in most yolky embryos intersegmental furrows appear only *late* in development (*Hemimysis*, *Nebalia*, *Mesopodopsis*, *Hanseniella*, etc.), and their absence in early stages in the anterior region (as well as elsewhere) can have little significance (see also p. 554).

(2) The statement that 'there is no specific evidence of the cephalization of primary post-oral somites, except in the case of the tritocerebral somite' is inaccurate. The antennal segment of *Peripatus* and the antennulary segment of the Malacostraca shift relative to the mouth so that they become secondarily pre-oral (see p. 521, and Manton 1934, text-figures 3*B* to 4*I*), and Tiegs (1947) has shown the antenna of *Pauropus* 'to arise a little behind the stomodoeum' and later to migrate to a pre-oral position, and that of *Hanseniella* (1940) to appear first postero-lateral to the position where the stomodoeum will later arise.

(3) The statement that 'the embryonic coelomic sacs of the first antennae, the pre-antennae, and the labrum are formed directly where they occur in the cephalic mesoderm, and give no evidence of having been drawn forward from behind the mouth' is untrue.

The solid antennulary somites of the Malacostraca are drawn forward from the blastoporal area, and the antennal somites of *Peripatopsis balfouri* are hollow before they have reached a pre-oral position, to take but two examples. The segmentation of the anterior parts of the forwardly shifting mesodermal bands is very uniform in arthropods, excepting the curious origin of the pre-antennulary somites in the Malacostraca (see Manton (1934) for a possible explanation of this).

(4) That 'coelomic sacs of the acronal region (his extended acron) so far as known, are best developed in the higher Arthropods, and thus do not appear to be primitive structures' needs no further comment (see above) beyond disagreement with the statement.

(5) 'The protocerebral and deutocerebral parts of the brain are always connected by pre-oral commissures, the only post-oral cerebral commissure being that of the cephalized tritocerebral ganglia.' Tiegs (1940) has drawn attention to the tritocerebral commissure being pre-oral in most Chilopoda, and post-oral in Scutigera alone. The position of a commissure is determined by its time of appearance relative to the shift of the mouth and segments, as pointed out by Heymons (1901). If the mouth passes backwards before the nerves develop the commissure may be pre-oral. The position of a commissure is thus of limited significance.

(6) to (8) These headings will not be discussed here as they do not appear to be at all conclusive.

Thus there is much evidence against Snodgrass's interpretation of the arthropod head, and even if there were evidence of weight in its favour this theory would create great difficulties when applied to the Onychophora and Arthropoda, as noted above. On the available evidence the older interpretation of head segmentation appears to be the more satisfactory. The uniformity of metamerism in Onychophoran embryos appears to be primitive, and the antennal segment appears to be the first segment of the body.

Arising out of Snodgrass's interpretation of arthropodan head structure and his suggested ectodermal origin of metamerism are many claims which will not receive support from workers on particular groups of arthropods. A few of these may now be mentioned. Concerning the crustacean nauplius, he relegates the anterior region including the antennulary segment to an 'acron', and describes the rest of the body as consisting of antennal and mandibular segments, an area which will give the maxillule and maxillary segments, and a telson at the base of which is a generative zone. He states that the nauplius therefore represents a stage with four primary somites (although the limbs on the posterior two are not yet formed); and he further considers that 'it would seem to be more than a coincidence that the same number of primary somites occurs in the Malacostraca, Xiphosurida and Trilobita'. Few carcinologists would accept such an interpretation of a nauplius. Sollaud's claim (1923) that in the Palaemonidae the maxillule and maxilla belong to the primary 'naupliar' segments has not been confirmed, and work with more satisfactory technique has shown these segments to be teleoblastic in origin in members of the Decapoda (*Caridina*), Peracarida (*Hemimysis*, *Mesopodopsis*), Hoplocarida (*Squilla*) and Leptostraca (*Nebalia*). There may be four naupliar or primary segments in the Crustacea, but they are the pre-antennulary, antennulary, antennal and mandibular segments, and not the regions given by Snodgrass.

Some of the characters supposed to be common to Annelida, Onychophora and Arthropoda cannot be substantiated. The statement that the preblastoporal region gives rise only to the prostomium and cephalic lobes is untrue, the anus as well as the mouth may be preblastoporal, as in some Onychophora and Crustacea (*Caridina*), see pp. 543 and 545. Snodgrass states that 'in its full development the arthropod mesoderm surrounds the blastopore anteriorly; since in the adult the lateral bands of cephalic mesoderm may be continuous from side to side in front of the mouth'. As the mouth and the blastopore are not necessarily the same thing in Arthropoda and Onychophora (see p. 541) this statement has little significance, and in any case the anterior union of the mesodermal bands is a secondary occurrence during development (see figures 6 and 7). His suggestion that the cephalic mesoderm is fundamentally radial and cannot represent somites as do the paired sacs posterior to the mouth, possibly arises out of the former consideration, but it does not appear to carry much in its favour. If applied to the Onychophora or Crustacea it raises difficulties rather than explains any.

Some of the views put forward by Snodgrass concerning Crustacea inspire little general acceptance. The origin and uses of the various types of limb in Crustacea is a functional problem on which much work has been done. The survey of structure of the limbs and head in Crustacea presented by Snodgrass is misleading and hardly represents our present knowledge of the subject.

The theory of annelidan and arthropodan head segmentation elaborated by Henry, Ferris and Hanke (1947 and 1948).

Since the present work was written my attention has been directed by Dr H. E. Hinton to a series of papers which have appeared on the head segmentation of Annelida and Arthropoda (Ferris 1947, 1948; Henry 1947, 1948; Hanke 1948), involving basic assumptions and the development of a theory opposed to anything which has hitherto been put forward. This work is founded upon a belief in a stability of the nervous system throughout large sections of the animal kingdom, and with the view that the nervous system represents 'the basic material for the determination of homology throughout the Annulata'. The data considered consist of details of the nervous system obtained by dissection of adult animals. The paths of the nerve tracts within the central nervous system have not been followed, no investigation of the development of nervous systems has been attempted, and evidence derived from other sources is mostly disregarded. Reliable work which increases our knowledge of comparative anatomy is valuable, and is particularly welcome concerning the nervous system of segmented invertebrates. However, in the papers referred to above, many unsubstantiated assumptions have been made in the development of a new set of theories. A full review of these theories will not be given here, but for the benefit of the general reader some of the major matters with which the writer of the present work is in disagreement are listed below.

The diagnosis of a segment has received considerable attention in earlier literature (and see pp. 555 and 556 above), and it is inconceivable that a segment can be defined upon the external form of the adult nervous system alone. In vertebrates experimental work has shown that mesodermal metamerism determines that of the nervous system (see p. 553),

and it is well known that a reduction or disappearance of a somite leads to the disappearance of the nerve components supplying it.

The identification of a prostomium depends upon the assumed nature of this structure, but, in the ordinary sense of the word, the recognition of an unsegmented region lying anterior to that divided into segments must ultimately be determined by embryology, since adult anatomy cannot provide the necessary data. If a true prostomium of considerable size exists in the adult state, this prostomium is likely to possess nerves, and such nerves can hardly be expected to arise from anything but part of the cerebral ganglion.

It should be borne in mind that the ontogenetic development of the nervous system in a clearly recognizable form usually takes place much later than that of the segmental mesodermal somites, muscles, limbs, etc., and in arthropods *after* extensive alterations in the relative positions of the mouth and associated structures are completed. However, cells destined to become nerve cells may be recognizable very early, as early as the mesodermal rudiments. In many animals it is clear that cells so predetermined multiply and develop in an orderly manner, but a full understanding of the process would involve tracing the lineage of enormous numbers of cells, and this would be a work of such magnitude that it has not yet been attempted.

(1) The denial of the occurrence of pre-oral segments in arthropods is contrary to clear embryological data (see p. 559 above).

(2) The evidence for the belief that the prostomium in Oligochaeta is homologous with the first body segment is based merely upon the occurrence of a varied series of nerves to this region from the cerebral ganglion. It would be remarkable to find a prostomium as large as it is in *Lumbriculus* with no nerves supplying it, and the occurrence of such nerves can hardly provide evidence for the interpretation offered.

(3) The assumption that the point of emergence of the stomodoeal system is 'a fixed landmark' in all groups is, in the absence of neurological evidence concerning the paths of nerve tracts, no more than an unproven suggestion. It should be remembered that the point of emergence of homologous nerves can vary greatly, for example the hypoglossal nerve may be cranial to brachial in emergence. The further argument, that the stomodoeal system always arises from the first pair of ganglia of the ventral chain, depends upon the above assumption, on the proximity of the points of emergence of theprostomial nerve and one of the stomodoeal connectives in Oligochaetes, etc., and on the view that this prostomium is the first segment of the body, and *therefore* the prostomial and stomodoeal nerves must arise from a first pair of ganglia. The view that the cerebral ganglion of Polychaeta is fused with the first pair of ganglia of the ventral chain (tritocerebral ganglia) follows on the same assumptions. Such reasoning cannot be seriously entertained.

(4) Similar evidence leads to the supposition that the crustacean tritocerebral ganglion belongs to the first segment of the whole body (including head). It would indeed be remarkable to find that this ganglion has secondarily migrated posteriorly in the Branchiopoda (alone among Crustacea), while the mesodermal somites, etc., with which it is usually considered to be associated are clearly seen to migrate forwards relative to the mouth (see also pp. 521 and 559).

(5) The view that the polychaete prostomium is born on segment three is unsubstantiated in the absence of embryological evidence, and the further claim that the jaws of

polychaetes are homologous with those of the Onychophora, and that both arise on the first segment, is based on the reasoning criticized above.

(6) The evidence for the existence of 'labral' and 'clypeal' segments in Crustacea is negligible. The statement that the conclusions reached concerning head segmentation in Crustacea 'are in accord, in principle, with the work of embryologists (Manton 1928)' is untrue. The work of Manton (1928, 1934) and the present account in no way supports this theory. The finding of five head segments in the Onychophora is based on a series of unwarrantable assumptions and does not merit serious consideration. Similar criticisms apply to conclusions concerning other groups.

It is clear that further embryological work is needed on the Annelida as well as the Arthropoda, and with particular reference to head segmentation and the phenomenon of primary and secondary metamerism. The development of the nervous system needs elucidation together with comparative work on nerve tracts in segmented invertebrates.

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Key to the lettering on the figures

a. anus.
a.e. external opening of anus.
a.g. anterior thickening of germinal disk, giving mouth-anus.
a.i. internal opening of anus.
*a.*1 antennulary segment.
*a.*2 antennal segment.
ar. archenteron.
B. almost virtual blastopore between edges of hemisphere of blastomeres
b. blastomere.
b.a. blastoporal area.
b.v. blastodermic vesicle, or yolkless yolk sac.
bl. complete blastoderm round the periphery of the embryo, formed from the saddle of blastomeres *sa.*
bl.e. part of blastoderm which is lined by endoderm and which persists after shrinkage of the blastodermic vesicle.
bl.s. part of blastodermic vesicle which is not lined by endoderm and which disappears.
bp. blastopore.
bp.e. edges of the blastopore.

c.m. cytoplasmic sheet lying between the lips of the mouth-anus.
c.s. cytoplasmic spheres which are non-nucleated and are separate one from another.
d.n. degenerating nucleus.
d.n.v. degenerating nucleus in the remains of the network formed by the large vacuolated cells.
d.y.s. dorsal yolk sac, composed of thin ectoderm lined by endoderm.
e. ectoderm.
e.e. edge of sheet of endoderm spreading outwards from the blastoporal area.
e.f. endodermal folds just within the lips of the mouth-anus.
en. endoderm.
e.v. embryonic vesicle.
f. darkly staining inclusion, probably food material.
*f.*48, *f.*52, etc., plane of section shown by figures 48, 52, etc.
g. germ cells.
g.b. thickened ectoderm of germinal band.
g.c. 'giant cell.'
g.d. germinal disk.
i.f. ingested food body taken up by blastomere from the non-nucleated cytoplasmic spheres.
i.m. inner embryonic membrane.
l. thickened lips of the mouth-anus or of its rudiment.
l.v.c. 'large vacuolated cell' formed by an edge cell of the saddle of blastomeres *sa.e.* becoming free.
lo. lower end of oviduct.
lo.d. dilatation occupied by an embryo in the wider lower oviduct.
m. middle line.
mdb. mandibular segment.
me. mesoderm.
mo. mouth.
mo.-a. mouth-anus.
*mx.*1 maxillulary segment.
n. network formed by degenerating remains of the 'large vacuolated cells'.
o.m. outer embryonic membrane.
o.r. optic rudiment.
p. nucleated cytoplasm of the egg which has separated from the nutritive part *y*.
*p.b.*1 and *p.b.*2, first and second polar body.
p.g. posterior thickening of germinal disk, forming blastoporal area.
p.l. protruding lips of mouth-anus.
pr. proctodoeal intucking.
r. vestigial rudiment of mouth-anus.
s. fluid filled space in which the cytoplasmic spheres *c.s.* and the blastomere *b.* float.
s.d. secretory droplet either in giant cell or derived from it.
s.x. abnormal extra pair of somites.
*s.*1, *s.*2, *s.*3, etc., first, second, third, etc., somite.
sa. saddle of blastomeres (on plate 39 up to stage *i*), and hemisphere of epithelial cells formed from the saddle in stage *j*.
sa.e. edge cell of saddle of blastomeres which has ingested food bodies from the cytoplasmic spheres and is becoming swollen and vacuolated.
st. stomodoeal ectoderm.
st.a. stalk of attachment.
sto. position where stomodoeum will be formed.
t. teloblastic area.
*t.f.*4 area shown in surface view in figure 4, p. 517.
t.bl. thin blastoderm with tangentially flattened nuclei formed by the dilatation of the blastodermic vesicle.
th.bl. thick blastoderm of the germinal disk.
u.c. undifferentiated cell of posterior thickening of the germinal disk.
u.m.b. posterior undifferentiated part of the germinal band mesoderm.
up. upper end of oviduct.
up.o. upper narrow section of oviduct.
v.b. thin ventral body wall formed from the fused part of the lips of the mouth-anus.
v.t. vesicular thickening.
v.y.s. ventral yolk sac formed by thin ectoderm lined by endoderm.
x. position occupied by main mass of germ cells in some embryos.
y. nutritive material of the egg which has separated from the protoplasmic portion, but the whole is coherent.
y.s. yolk sac.
yo. yolk.

Description of plates 31 to 41

Plate 31

Embryos of *Peripatopsis sedgwicki* approximately as they appear in life by reflected light. The more opaque thicker tissues are lightly shaded and the transparent thin tissues are dark, but the ordinary shading effects of a solid object are superimposed on the above convention. The letters *B'*, *C'*, etc. denote developmental stages, and figures of the same stages on other plates, either as sections or reconstructions, bear the same letters. × 33 approx.

Figure 8. Embryo just beyond the end of segmentation. The inner membrane has just been absorbed, but the second dilatation has not yet taken place, and the dimensions of the embryo are the same as those on figures 17 to 20, plate 32. A germinal disk has appeared at one pole bearing two internal cells.

Figure 9. Embryo older than the last with dilating blastodermic vesicle and larger germinal disk. No mouth-anus is present. The outer membrane is much thinner as a result of dilatation, and lies tightly over the blastodermic vesicle and has not been drawn separately. A reconstruction is shown in figure 21, plate 32.

Figure 10. Embryo older than the last in lateral view with open mouth-anus, mesodermal bands present and first somite differentiating (see reconstruction in figure 23, plate 32). The anterior thickening of the germinal disk lies on the left and the posterior thickening on the right. The germinal disk is becoming concave towards the anus, not visible here.

Figure 11. Oblique view of embryo with mouth and anus and about ten mesodermal somites differentiated. Only two segments are externally visible by the ectodermal bulges. Further dilatation of the blastodermic vesicle has occurred, and the flexure of the definitive embryonic rudiment reveals the stretched outer membrane where it lies away from the embryo. The thin ventral wall of the yolk sac is visible. A reconstuction is shown in figure 26, plate 32.

Figure 12. Lateral view of embryo with twenty somites, 6 segments being externally demarcated. The coiling of the germinal bands is in progress, the mid-ventral distance between mouth and anus being much shorter than the length of the germinal bands between these levels.

Figure 13. Embryo with all somites formed, and the dilatation of the yolk sac now at a maximum. The dorsal and ventral walls of the yolk sac behind segment 7 are narrow. Limb bulges are forming anteriorly, and the rudiment of the antenna is apparent.

Figure 14. Older embryo with shrinking yolk sac now attached dorsally by a narrow neck. The body shows two main flexures and the limb rudiments are well formed.

Figure 15. Embryo a little older than the last, and removed from its embryonic membrane. The lips are formed and the jaw has passed into the oral cavity, leaving the slime papilla visible in lateral view.

Plate 32

The capital letters denote similar stages shown on other plates. Figures 16 to 20 show segmentation stages in which the blastomeres lie against the embryonic membrane and gradually surround a fluid-filled internal space (here appearing dark), in which float cytoplasmic spheres of various sizes. These are visible in life, but their numbers and positions have been reconstructed from sections and incorporated into the figures. Conventions as on plate 1. The outer membrane is thick and lightly shaded, and the thin inner membrane is shown by a black line in *P. moseleyi*, but the two membranes are indistinguishable in the whole embryo of *P. sedgwicki*, figure 20. Figures 21 to 26 represent reconstructions of germinal disks of *P. sedgwicki*, and a key to the conventions is given on plate 34. Figures 16 to 19 are of *P. moseleyi* and figures 20 to 26 are all figures of *P. sedgwicki*, × 140 approx.

FIGURE 16. *P. moseleyi*, 2-cell stage. The two blastomeres lie at one side of the membrane; they show numerous chromosome spheres instead of a single nucleus. Several cytoplasmic spheres of large size float in the fluid-filled internal space. See transverse section in figure 51, plate 35.

FIGURE 17. *P. moseleyi*, 21-cell stage. All the blastomeres are visible and form a superficial saddle on one side, no blastomeres being present on the other side of the embryo. A few cytoplasmic spheres float in the internal space.

FIGURE 18. *P. moseleyi*, 53-cell stage in lateral view. Half of the blastomeres are visible here, the saddle of blastomeres wrapping round two-thirds of the embryo. The cytoplasmic spheres are still present. See transverse section in figure 52, plate 35.

FIGURE 19. *P. moseleyi*, about 215-cell stage. The saddle of blastomeres has spread to form a continuous blastoderm everywhere, and situated just within the membrane. All blastomeres are alike and are tangentially flattened, unlike their form in earlier stages. All cytoplasmic spheres have been absorbed. The inner membrane has been absorbed and dilatation is about to take place. See transverse section in figure 53, plate 35.

FIGURE 20. *P. sedgwicki*, 55-cell stage, which is intermediate between those in figures 18 and 19 for *P. moseleyi*. The saddle of blastomeres, seen in the 53-cell stage of *P. moseleyi* (figure 18), has spread right round the middle of the embryo forming a cylinder with open ends, and of similar appearance on all sides. Cytoplasmic spheres and loose masses of cytoplasm lie within the cylinder, and at either end extend almost to the egg membrane at the poles.

FIGURE 21. *P. sedgwicki*, reconstruction of the germinal disk of the stage shown in figure 9, plate 31. A key to the conventions is given on plate 4. The limits of the disk are sharply demarcated by thickened ectoderm (pale grey). The endoderm immigrating from the part of the blastoporal area which is vertically hatched has spread under the whole disk, and is thickened below the future mouth-anus ectoderm (radiating lines) but the two layers are here separate. The zone of mesodermal immigration (horizontal hatching) is small, and the mesoderm forms a compact undifferentiated mass (dark shading). See transverse section in figure 55, plate 36, at the level indicated.

FIGURE 22. *P. sedgwicki*, reconstruction of the germinal disk at a stage intermediate between those shown in figures 9 and 10, plate 31. The disk is larger (the lateral parts are not shown). The ectoderm and endoderm are now united at the sides of an unperforated mouth-anus rudiment (heavy sagittal line). The blastoporal area is larger (hatched), the endoderm has spread a little beyond the disk, and the enlarged mesodermal mass has formed a small genital rudiment (heavy dots (*g.*)). See transverse section in figure 56, plate 36, at the level indicated, and longitudinal section in figure 54, plate 35, and figure 4, p. 517, showing the zone enclosed by dotted line (*t.f.* 4).

FIGURE 23. *P. sedgwicki*, reconstruction of the germinal disk at the stage shown in figure 10, plate 31. The disk is about the same size as in figure 22. The mouth-anus is now opening and is traversed by a few strands of protoplasm. Endoderm now lines the whole blastodermic vesicle. The area of mesodermal immigration is more extensive, and mesodermal bands are growing forwards on either side of the mouth-anus from the undifferentiated posterior mass. The genital rudiment is larger.

FIGURE 24. *P. sedgwicki*, reconstruction of the germinal disk at a stage intermediate between that shown in figures 10 and 11, plate 31. The mouth-anus has divided to form a large mouth and a minute anus, of the latter the external opening only is shown (see figure 25 for internal opening of the anus). Both mouth and anus are completely open. The mesodermal bands have formed three pairs of hollow somites, and a solid fourth pair is indicated but not separate from the band. The blastoporal area is now longer and narrower, and the genital rudiment larger and asymmetrical.

FIGURE 25. *P. sedgwicki*, reconstruction of a germinal disk older than the last and younger than that shown in figure 11, plate 31. The germinal disk is considerably larger but the extremities of the mouth-anus are the same distance apart as in figure 24, the mouth is smaller. The germinal bands now bow outwards, and the thin-walled zone (*v.y.s.*) has appeared on each side. Coelomic cavities lie in the first five pairs of somites, and the rudiments of eight somites are differentiated. The genital rudiment has enlarged. The disk is no longer flat (see figure 11, plate 31).

FIGURE 26. *P. sedgwicki*, reconstruction of the germinal disk of the stage shown in figure 11, plate 31. Endodermal immigration has ceased. The germinal bands now bow farther out, the mouth and anus have drawn farther apart, and the thin-walled zones (*v.y.s.*) have united mid-ventrally. Somite formation has progressed, and part of the genital rudiment has shifted backwards in an irregular manner.

PLATE 33

Embryos of *Peripatopsis moseleyi* approximately as they appear in life by reflected light. The more opaque thicker tissues are lightly shaded and the transparent thin tissues are darker, but the ordinary shading effects of a solid object are superimposed on the above convention. All are enclosed by the outer membrane only, the inner membrane having been absorbed. The letters *E*, *F*, etc., denote developmental stages, and figures of the same stages on other plates bear the same letters. × 33 approx.

FIGURE 27. Embryo before the second dilatation has taken place; its dimensions are the same as at the end of segmentation, figure 19, plate 32, the outer membrane is conspicuous and the inner membrane has been absorbed. A germinal disk lies midway between the poles of the embryo and consists of about nine external cells and two that have immigrated to the interior. See longitudinal section shown in figure 48, plate 35.

FIGURE 28. Dilatation of the blastodermic vesicle has started and the germinal disk is larger, appearing as an opaque spot in life; it consists of the posterior thickening alone. The endoderm has spread to the margin of the disk. The stretched outer membrane is not visible here. For reconstruction see figure 39, plate 34 and for longitudinal section see figure 49, plate 35.

FIGURE 29. Dilatation of the vesicle continues, the germinal disk has enlarged, and its anterior thickening is detectable in sections (see reconstruction in figure 40, plate 34), although not visible externally. The pale area extending round the germinal disk (*en.*) represents the extent of the spreading endodermal layer which renders the walls of the blastodermic vesicle more opaque. Mesoderm and genital rudiment lie under the germinal disk. See longitudinal section of a slightly younger stage in figure 49, plate 35.

FIGURE 30. Embryo slightly older than the last with well-developed anterior thickening which is only slightly smaller than the posterior thickening. See reconstruction in figure 41, plate 34.

FIGURE 31. Embryo slightly older than the last. The posterior thickening of the germinal disk is much larger than the anterior thickening, the mouth-anus rudiment is well formed, and a transparent line (dark) is seen where the lip edges are apposed. Endoderm now lines the whole embryo. See reconstruction in figure 42, plate 34.

FIGURE 32. Dilatation of the blastodermic vesicle or yolk sac is now almost completed. The closed mouth-anus is longer, showing thickened opaque (light) lips. The posterior thickening has formed the germinal bands, which have spread alongside the lips of the mouth-anus, and the first pair of somites are differentiated. No segmental differentiation of the ectoderm is present, the first somites are visible by the greater transparency where there is a break in the mesoderm. See reconstruction in figure 43, plate 34, and transverse sections in figures 57 and 59, plate 36.

FIGURE 33. Dilatation of the blastodermic vesicle or yolk sac is now at a maximum. The germinal bands have bowed outwards leaving the thin ventral wall of the yolk sac between them and the thickened lips of the mouth-anus on either side. The first pair of somites are approximating in front of the mouth-anus. Four pairs of mesodermal somites have separated from the bands. See reconstruction in figure 44, plate 34.

FIGURE 34. Older embryo in which the mouth and anus have separated, but they are still closed. The germinal bands have elongated considerably although the mid-ventral distance between the mouth and anus is the same as the length of the undivided mouth-anus. Seven mesodermal somites lie in the band anterior to the main flexure, and eight hollow and one solid somites lie in the band posterior to the flexure. The level of the blastoporal area from which immigration of mesoderm is proceeding is indicated (*b.a.*). The thin-walled ventro-lateral regions seen in the previous stage have united to form the enlarging ventral wall of the yolk sac. The stretched outer membrane is visible on the right. See reconstruction in figure 45, plate 34.

FIGURES 35 and 36. Lateral and oblique views of the same embryo with about eighteen somites. The mouth and anus are farther apart, the ventral wall of the yolk sac has enlarged, and the germinal bands are longer and have bowed farther out. Limb bulges are appearing anteriorly. The position of the elongated blastoporal area is shown in figure 35, *b.a.* A reconstruction of a slightly older stage is shown in figure 46, plate 34.

FIGURE 37. All somites have just been formed. The germinal bands have enlarged at the expense of the yolk sac which is now smaller. The definitive head region is bent downwards and foreshortened. The first segment shows the ventral brain and the dorsal antennal rudiments. The jaw has already shifted inwards, and is covered by the antenna. The rounded slime papilla is conspicuous. The dorsal extents of the anterior somites are seen on the right; behind somites 12 they nearly meet dorsally, and are separated by only a narrow tract of yolk sac. Ventrally the germinal bands are separated by a wide ventral wall of the yolk sac. Limb rudiments can be seen as far as segment 15. The body here curls to the left, but this is a variable feature. The mouth and anus are farther apart than in the last stage (see reconstruction in figure 47, plate 34), but the mid-ventral distance between them is much shorter than the corresponding lateral distance along the bands.

FIGURE 38. All somites are formed, and the mid-ventral distance between the mouth and anus has increased so that the anus is now terminal. The body shows two main flexures. The somites from the seventh pair backwards almost meet dorsally so that the shrinking yolk sac is mainly restricted to the region anterior to this level. The ventral wall of the yolk sac is now a narrow tract, visible as a dark mid-ventral line owing to its transparency. Ventral to the antenna can be seen the brain rudiment. The lateral lips of the mouth are visible between the antenna and the slime papilla; the limb rudiments project from nearly all segments, and the rudiments of the nerve cords lie as opaque bands between the limbs and the reduced ventral wall of the yolk sac.

PLATE 34

Reconstructions of the germinal disks of a series of embryos of *P. moseleyi*. The letters *F*, *G*, etc., denote stages shown on plate 33, and the arrows *f.* 49, etc., show the planes of sections shown on other figures. Figures *F* to *L* × 140 approx., figures *M* to *nrO* × 68 approx.

Key to the conventions used here and on plates 32, 38 *and* 41.

A. Ectoderm of the germinal disk which is thicker than that of the rest of the embryo (white), into which it gradually merges.

B. Mesoderm, either as a solid band or as the wall of a hollow somite.

C. The dark shading represents a somite wall in optical section, the white space being the coelomic space.

D. Undifferentiated genital rudiment.

E. Groups of germ cells or single germ cells which have separated from the original rudiment, and which lie between the ectoderm and the endoderm.

F. Each black rectangle represents a single germ cell which has migrated into the wall of a somite. (The rectangles shown in the key are obscure in the reproduction, but they are plainly seen in figures 46 and 47.)

G. The vertical line represents the mouth-anus rudiment with closed lips, the ectoderm and endoderm being continuous on either side at the lip edges. The radiating lines represent thickened endoderm of the lips.

H. Mouth-anus open, except for a few strands of protoplasm traversing the opening.

I. Completely open mouth or anus.

J. Part of blastoporal area where endodermal cells are immigrating inwards, vertical hatching.

K. Part of blastoporal area where mesodermal cells are immigrating inwards, horizontal hatching.

L. Part of blastoporal area where the endodermal and mesodermal immigrations cannot be differentiated.

FIGURE 39. Embryo at the stage shown in figure 28, plate 33. The posterior thickening (*p.g.*) is present alone. The endodermal immigration has formed about twenty-three endodermal cells which lie under the germinal disk and just beyond it (see longitudinal section, figure 49, plate 35). The genital rudiment comprising about seventeen cells, but no mesoderm, lies under the disk.

FIGURE 40. Embryo at the stage shown in figure 29, plate 33. The anterior thickening of the germinal disk is now present (*a.g.*) on which is forming the mouth-anus rudiment; the endoderm extends far beyond the disk (see the whole view, figure 29, plate 33). The ectodermal limits of the disk are not clearly defined. The mesodermal immigration has given rise to a disk of mesoderm lying internal to and beyond the genital rudiment.

FIGURE 41. Embryo at the stage shown in figure 30, plate 33, in which the anterior thickening of the germinal disk is almost as large as the posterior thickening. The mouth-anus rudiment is larger than in figure 40, but otherwise this embryo is essentially the same as the last although the proportions are different.

FIGURE 42. Embryo at the stage shown in figure 31, plate 33, older than the last. The mouth-anus rudiment is larger; the posterior thickening and the internal mesodermal mass are larger, and the blastoporal area forms an elongated narrow zone. Endoderm lines the whole embryo.

FIGURE 43. Embryo at the stage shown in figure 32, plate 33. The mouth-anus rudiment is now fully formed and at its maximum length. Mesodermal bands have spread forwards lateral to the mouth-anus; the first pair of somites have separated from the band, and the cavities of the second pair are forming from several initial spaces. The genital rudiment is irregular in outline. See transverse sections in figures 57 and 59, plate 36.

FIGURE 44. Embryo at the stage shown in figure 33, plate 33. The germinal bands have started to bow outwards, so leaving the thin ventral wall of the yolk sac (*v.y.s.*) on either side. Four pairs of somites have separated from the bands, and a fifth pair is differentiating.

FIGURE 45. Embryo at the stage shown in figure 34, plate 33. The mouth and anus are now separated, but their extreme ends are the same distance apart as are the ends of the mouth-anus in the last figure. The germinal bands have elongated considerably, and fifteen hollow and one solid pair of somites have been formed. Most of the genital rudiment has shifted to a position between the dorsal ends of the posterior somites, lying in a compact mass and in many small groups of cells, and about twelve germ cells remain on either side of the blastoporal area.

FIGURE 46. Embryo a little older than the stage shown in figures 35 and 36, plate 33. The mid-ventral body wall is the same length as before, but the lateral germinal bands have elongated further, and nineteen hollow mesodermal somites have been formed. The genital rudiment is now broken up into many portions, most of which lie dorsally between the somites. Three germ cells have penetrated into the somite walls (black rectangles). Endodermal immigration has just ceased, and the mouth and anus have opened. See longitudinal section in figure 58, plate 36.

FIGURE 47. Embryo a little older than the stage shown in figure 37, plate 33. All somites are formed (twenty-seven in number). The mid-ventral body wall between mouth and anus has elongated, and the lateral germinal bands are very much longer. The dorsal and ventral walls of the yolk sac in the posterior twelve segments is very narrow, and this part of the body is coiled. Somites 1 to 9 are seen in ventral view, somites 14 to 20 are seen in dorsal view, somites 21 to 24 are seen approximately laterally, and the posterior end of the body obliquely covers the anus, which is indicated by dotted lines. Where somites overlap one another in this view the outlines of those farther away are omitted. Immigration of mesoderm has just ceased, and the remains of the mesodermal bands unite the last pair of somites, the cavities of which are just forming. The genital rudiment is entirely broken up into separate cells and small groups. Twelve free germ cells lie in front of the anus, eight lie elsewhere, and thirty have become incorporated into somite walls, twenty-two of these being shown by black rectangles (the others lie in the somite walls omitted from the figure).

PLATE 35

FIGURE 48. *P. moseleyi*, section longitudinal to the main axis of the blastodermic vesicle of the embryo shown in figure 27, plate 33. The inner membrane has just been absorbed, but the second dilatation has not yet taken place. The section shows the only two internal cells of the germinal disk. Thin blastoderm, like that shown on the right, forms the rest of the vesicle which is omitted from the right side of the figure. The internal cell (*en.*) has become endoderm, and the other is not yet histologically differentiated. × 450 approx.

FIGURE 49. *P. moseleyi*, sagittal section of the germinal disk of the stage shown in figure 28, plate 33, and reconstructed in figure 39, plate 34. Endoderm lies under the whole disk, and numbers twenty-three cells (*en.*). The blastoporal area (*b.a.*) is shown, and the genital rudiment (*g.*), consisting of seventeen cells, lies between the blastoderm and the endoderm, cell boundaries are not visible here. No mesoderm has yet been formed. × 450 approx.

FIGURE 50. *P. moseleyi*, slightly oblique sagittal section of a slightly older embryo. In front of the blastoporal area the section does not cut the middle line, but no mouth-anus is yet clearly defined. The germinal disk is larger, and the ectodermal thickening extends far beyond the field of view. The endodermal cells have enlarged and spread beyond the disk. The genital rudiment is larger and may be increasing by immigration as well as by mitosis. The first mesodermal cells are probably present just within the blastoporal area, but they cannot be differentiated from the immigrating endoderm. × 450 approx.

FIGURE 51. *P. moseleyi*, transverse section of the 2-cell stage shown in figure 16, plate 32. The outer membrane has been removed and the inner one is figured. One blastomere is cut through the middle showing the form of the nucleus in many separate chromosomal vesicles, and the other is cut through the edge of the nuclear zone. Few cytoplasmic spheres lie in the central space, they may be larger and more numerous in other embryos. × 350 approx.

FIGURE 52. *P. moseleyi*, transverse section of a 52-cell stage, see the 53-cell stage shown in figure 18, plate 32. The outer membrane has been removed. The section passes through one of three large cytoplasmic spheres (*c.s.*); and the saddle of blastomeres (*s.a.*) lies on one side. Poor fixation of the nuclei is due to fixation in Duboscq-Brazil, but the lobed form of some of the nuclei is no artefact. × 350 approx.

FIGURE 53. *P. moseleyi*, transverse section of an embryo slightly older than that shown in figure 19, plate 32. Dilatation has started, but no germinal disk has appeared. The inner membrane has been absorbed and the outer one has been removed. The whole blastodermic vesicle contracted slightly on fixation. The blastoderm is of even thickness, no internal cells are present, and the last remains of the cytoplasmic spheres can be seen. × 350 approx.

FIGURE 54. *P. sedgwicki*, longitudinal section of a stage intermediate between those shown in figures 21 and 22, plate 32. The section is almost sagittal, passing through edges of the lip of the mouth-anus (*l.*), rich in mitoses, and showing union of ectoderm with endoderm. The blastoporal area *b.a.* shows immigrating cells, which merge anteriorly into the endodermal layer extending anteriorly and posteriorly, and into the mesodermal mass posteriorly (*me.*). Elsewhere both endoderm and mesoderm are separate from the overlying blastoderm. The first two germ cells to be differentiated from the mesoderm are cut (*g.*). Anteriorly the transition to the thin blastoderm of the vesicle is seen. (N.B. the limits of the blastoporal area and the mouth-anus rudiment can only be determined clearly by reference to a series of sections.) × 450 approx.

PLATE 36

The formation of the mouth-anus is shown by the transverse sections in figures 55 to 57. All figures × 450 approx.

FIGURE 55. *P. sedgwicki*, transverse section of the stage reconstructed in figure 21, plate 32, at the level indicated. The endoderm (*en.*) is thickened near the middle line (marked *m.*), but is quite separate from the ectoderm. The outer membrane is closely adherent to the ectoderm.

FIGURE 56. *P. sedgwicki*, transverse section of the stage reconstructed in figure 22, plate 32, at the level indicated. The mouth-anus rudiment is further advanced. Both ectoderm and endoderm show orientated nuclei on either side of the middle line (*m.*), giving continuity of the ectoderm and endoderm on either side at the future lip edges (*l*). Two mitoses, of the many occurring in the lips, are cut here. Laterally the ectoderm and endoderm are clearly separate.

FIGURE 57. *P. moseleyi*, later stage with the mouth-anus established, see the reconstruction in figure 43, plate 34, on which the level is indicated. The section passes through the fully formed lips (*l.*) of the mouth-anus, which in this species are apposed for a considerable period. The exact junction between ectoderm and endoderm in the lips is not determinable here, although plainly visible in *P. balfouri*, see figures 110 and 111, plate 40. The first somite (*s.* 1) is cut on the left, and its coelomic space is forming.

FIGURE 58. *P. moseleyi*, longitudinal section through a stage resembling that reconstructed in figure 46, plate 34, at the level indicated, and illustrating the migration of the germ cells into the somite walls. Somites 15 to 19 are cut, and the cavity of somite 19 is just appearing. The germ cell by somite 17 is pressing into the wall from the outside, and that by somite 18 has become part of the wall (see black rectangles in figure 46). Other germ cells lie elsewhere between the somites and the endoderm, or between ectoderm and endoderm far from the somites, and do not press into the walls of the latter.

FIGURE 59. *P. moseleyi*, transverse section of the stage reconstructed in figure 43, plate 34, at the level indicated. From the blastoporal area (*b.a.*) mesoderm (*me.*) is streaming inwards, and between the mesoderm and the endoderm lies the genital rudiment (*g.*), which has been cut twice in this section.

PLATE 37

A series of stages of *P. balfouri* is shown on plates 37 and 38 approximately as they appear in life, the more opaque blastomeres, etc., being light and the transparent fluid filled spaces being dark, as on plates 32 and 33. After a coherent embryo is formed, the space between it and the membrane is left white (figure 69 onwards), although it appears much as does the fluid filled space of earlier

stages which is represented by dark shading. The inner (*i.m.*) and outer (*o.m.*) membranes, which are about equal in thickness, are represented by a black line and by shading respectively in figures 60 to 68. Both are present up to figure 70, and thereafter the outer one alone is present. A series of reconstructions of many of these stages is shown, which bear the same letters (*l.*, *m.*, etc.) as the corresponding whole views. The conventions used are the same as on plates 32 and 34, a key to which is given on plate 34, the 'giant cell' is here shown by a finely dotted area. × 120 approx.

FIGURE 60. The earliest stage obtained from the oviduct, an unsegmented egg enclosed by both membranes and showing two polar bodies. The egg substance is coherent, and the nucleus lies in a lateral mass of cytoplasm (*p.*) which merges into a finely granular mass of possibly nutritive material (*y.*). See sections at the levels indicated in figures 97 and 98, plate 39.

FIGURE 61. An abnormal egg which will develop no further, showing the normal break up of the egg substance into separate cytoplasmic spheres floating in a fluid content of the membranes, but the chromatin is lodged in three of these spheres, two being visible in this view, and in all the chromatin is degenerating.

FIGURE 62. A normal egg showing the break up into a nucleated 'first blastomere' (*b.*) and many non-nucleated cytoplasmic spheres (*c.s.*) floating in the fluid filled space (*s.*) within the membranes. See transverse section in figure 99, plate 39.

FIGURE 63. A 6-cell stage. The blastomeres lie superficially against the membrane, and cytoplasmic spheres form a fairly dense zone almost filling the membrane.

FIGURE 64. An 8-cell stage similar to the above in general characters.

FIGURE 65. A 16-cell stage. The blastomeres are close under the membrane on one side, and the cytoplasmic spheres are a little smaller and more diffuse than before. See transverse section in figure 100, plate 39.

FIGURE 66. A 23-cell stage, resembling the last in general characters, but some of the non-nucleated cytoplasmic spheres are large and conspicuous, and the blastomeres are less regularly arranged.

FIGURE 67. A 39-cell stage, in which the saddle of blastomeres is more irregular, and few large cytoplasmic spheres float in the fluid filled internal space.

FIGURE 68. A 104-cell stage. A saddle of eighty-nine epithelial blastomeres lies on the side of the embryo in view. The edge cells of the saddle are swelling, and 15 have separated and lie among the cytoplasmic spheres on the other side of the embryo. No cytoplasmic spheres lie towards the poles. See transverse sections in figures 101 and 102, plate 39, at the levels indicated.

FIGURES 69 and 70 are of the same 113-cell embryo viewed from opposite sides. See transverse section in figure 103, plate 39.

FIGURE 69. The edges of the saddle of blastomeres seen in figure 68 have curled inwards, so that a hollow hemisphere is formed, seen in surface view in this figure, and consisting of 68 epithelial cells.

FIGURE 70. The same embryo viewed from the opposite side showing the concavity of the hemisphere seen in figure 69 filled with 45 large vacuolated cells (*l.v.c.*) derived from the saddle edges, only the outer of these cells are visible. The embryo is now a coherent, almost solid object, and the fluid space in which it floats is left white.

FIGURE 71. An embryo a little older than the last. The hemisphere of epithelial blastomeres seen in figure 69 has grown over the large vacuolated cells to form a continuous blastoderm on all sides. The inner membrane has been absorbed, and the blastodermic vesicle and the outer membrane have enlarged. The separate blastomeres are no longer drawn. See transverse section in figure 104, plate 39.

FIGURE 72. An embryo a little older than the last. The blastodermic vesicle and outer membrane have dilated to a maximum size, and the anterior and posterior thickenings of the germinal disk (*a.g.*) and (*p.g.*) have appeared. See reconstruction in figure 73 and transverse section in figure 105, plate 40.

FIGURE 73. Reconstruction of the germinal disk of the last embryo. The giant cell (*g.c.*) forms a large part of the posterior thickening, and the anterior and posterior thickenings are separate. See transverse section in figure 105, plate 40.

FIGURE 74. Reconstruction of the germinal disk of an embryo a little older than the last. Endoderm has immigrated inwards from the posterior thickening, and extends as far as the dotted line, see transverse section in figure 106, plate 40. The anterior thickening is more pronounced.

FIGURE 75. Reconstruction of the germinal disk of the embryo shown in figure 76. An open mouth-anus (*mo.-a.*) has been formed on the anterior thickening (unshaded). Endoderm has spread further outwards, mesoderm (*me.*) is present internal to the posterior thickening, and a blastoporal area (*b.a.*) is now clearly defined. See transverse sections in figures 108 and 109, plate 40.

FIGURE 76. Embryo with a more advanced germinal disk than that in figure 72. The anterior and posterior thickenings have almost united, and the former has given rise to a mouth-anus, closed by a delicate sheet of cytoplasm. Endoderm lies below and beyond the germinal disk under the more opaque (whiter) area, see reconstruction in figure 75.

FIGURE 77. Older embryo in which shrinkage of the blastodermic vesicle is almost complete. The germinal disk and the surrounding blastoderm lined by endoderm (figure 76) has become almost spherical (*bl.e.*), and the remaining thin blastoderm forms a shrunken dorsal remnant (*bl.s.*). The lips of the mouth-anus are now almost apposed, but the lumen is open.

PLATE 38

Continuation of the series of whole views and reconstructions of *P. balfouri* seen in plate 37. The outer membrane is shown in figures 78 and 95 but is omitted from the intervening stages. Unless otherwise stated, the embryos are seen in approximately ventral view. × 120 approx.

FIGURE 78. Slightly older embryo than the last. The shrinkage of the blastodermic vesicle is complete, resulting in a small, opaque, spherical embryo with a narrow open mouth-anus floating within the large outer membrane. See reconstruction in figure 79 and sagittal section in figure 110, plate 40.

FIGURE 79. Reconstruction of a stage similar to figure 78. The mesoderm forms a compact disk internal to the blastoporal area. Endodermal immigration has either ceased or is very slight. See sagittal section in figure 110, plate 40.

FIGURE 80. Embryo a little older than that of figure 78, with a larger mouth-anus, and a slight antero-posterior elongation of the body. See reconstruction in figure 81 and sagittal section in figure 111, plate 40.

FIGURE 81 Reconstruction of the last. Mesodermal bands have started to grow forwards on either side.

FIGURE 82. An older embryo with a more elongated mouth-anus.

FIGURE 83. An older embryo where the mouth anus has divided into an open mouth and anus. See reconstruction in figure 84.

FIGURE 84. Reconstruction of the last. The mesodermal bands have extended as far as the mouth, and the coelomic cavities of the first pair of somites have appeared.

FIGURE 85. An almost lateral view of an older embryo. The body has elongated, but not the mid-ventral distance between mouth and anus. The germinal bands (lightly shaded) are now differentiated from thinner dorsal and ventral walls of the yolk sac (darkly shaded), which here are far less extensive than in the other species. See reconstruction in figure 86.

FIGURE 86. Reconstruction of the last. The mesodermal bands have segmented into four separate hollow somites, and a fifth is indicated, but not yet separated from the band.

FIGURE 87. Reconstruction of an embryo at about the same stage as the last, but the details of the mesoderm are slightly different. Five hollow somites are formed, but most of them are still united, and the mesodermal bands extend horizontally outwards from the blastoporal area.

FIGURE 88. Older embryo in which the germinal bands have bowed outwards with their increase in length, so widening the thin ventral wall of the yolk sac (darkly shaded as it is more transparent). The dorsal wall is similarly widened. The mid-ventral body wall has elongated slightly.

FIGURE 89. Reconstruction of the last. Five pairs of separate, hollow, mesodermal somites are formed, the first pair being in front of the mouth. The ventral wall of the yolk sac (*v.y.s.*) is at its maximum width.

FIGURE 90. Older embryo in which the mid-ventral body wall has elongated, so straightening the germinal bands, and resulting in a narrowing of the ventral and dorsal walls of the yolk sac. See reconstruction in figure 91, and sagittal section in figure 112, plate 40.

FIGURE 91. Reconstruction of the last showing the shortened mesodermal bands and eight pairs of separate, hollow somites. A genital rudiment (not shown) lies internal to the blastoporal area, and has been differentiated from the mesoderm, see sagittal section in figure 112, plate 40.

FIGURE 92. Oblique view of an older embryo with about ten pairs of somites in which a ventral curvature of the body has started. The enlarging anterior mesodermal somites cause segmental bulges to appear externally in the ectoderm. The anus is narrow, and the position of the blastoporal area behind it is indicated (*b.a.*).

FIGURE 93. Oblique view of an older embryo with about eleven or twelve pairs of somites. The flexure of the body has increased, and the anterior somites have enlarged, the first pair being now closely pressed together in front of the mouth.

FIGURE 94. Lateral view of an older embryo with about nineteen or twenty pairs of somites. The levels of the mouth, anus, and blastoporal area are indicated, although invisible at this angle. The ventral wall of the yolk sac is seen, and a portion of the dorsal wall appears dorsal to the second somite. The ventral flexure has increased.

FIGURE 95. Older embryo with twenty pairs of somites, which now fills its membrane. The ventral wall of the yolk sac is narrower than in earlier stages seen in figures 88 to 91.

FIGURE 96. An older embryo removed from the outer membrane. All twenty-one pairs of mesodermal somites are formed, and mesodermal immigration has ceased. The body shows two main flexures. Limb rudiments are projecting externally. The ventral and dorsal walls of the yolk sac are narrower than before (see figures 88, 90 and 95). The genital rudiment has broken up into separate germ cells which lie in the walls of somites 15 to 20, none remaining free.

PLATE 39

Sections of *P. balfouri*, magn. ×350 approx.

FIGURES 97 and 98. Transverse sections through the unsegmented egg shown in figure 60, plate 37, at the levels indicated. Inner and outer membranes cannot be differentiated in this section (see p. 492). Cytoplasm (*p.*) is indicated by dark shading, and the unstained nutritive mass (*y.*) by a mechanical tint.

FIGURE 97. Passes through the nucleus lying in the lateral mass of cytoplasm (*p.*).

FIGURE 98. Passes through the second polar body (*p.b.*2) and the first polar body (*p.b.*1) is shown from a neighbouring section.

FIGURE 99. Transverse section of the unsegmented egg shown in figure 62, plate 37, in which the egg substance has broken down into a nucleated sphere of finely granular cytoplasm, the first blastomere (*b.*), which lies against the membrane, and a number of non-nucleated cytoplasmic spheres (*c.s.*), many of which contain darkly staining food inclusions (*f.*).

FIGURE 100. Transverse section of the 16-cell stage shown in figure 65, plate 37, at the level indicated. In general features it resembles the last, but the inner parts of the blastomeres contain ingested food inclusions (*i.f.*) from the cytoplasmic spheres.

FIGURES 101 and 102. Transverse sections of the 104-cell stage shown in figure 68, plate 37, at the levels indicated. The nuclei of the blastomeres do not all lie in these two sections but in neighbouring ones, thus some blastomeres appear to lack a nucleus and so resemble a cytoplasmic sphere, for example the left blastomere (*sa.e.*) in figure 101. The marginal blastomeres, and the inner parts of some others contain many ingested food inclusions (*i.f.*)

FIGURE 101. Passes through the middle of the saddle of blastomeres and shows the marginal blastomeres (*sa.e.*) which are starting to swell, and are rich in food inclusions, and a few cytoplasmic spheres lying in the fluid-filled space.

FIGURE 102. Passes through one end of the saddle of blastomeres, and shows three free swollen cells (*l.v.c.*) which have separated from the saddle edge, and lie among the many non-nucleated cytoplasmic spheres (*c.s.*). On the left the edge blastomere of the saddle (*sa.e.*) is much swollen and vacuolated preparatory to its separation. (N.B. complete reconstructions of the embryo are needed to ascertain which structures are non-nucleated cytoplasmic spheres and which are nucleated large vacuolated cells.)

FIGURE 103. Section of the 113-cell stage shown in figures 69 and 70, at the level indicated. The blastomeres of the saddle seen in figure 101 now form an epithelial hemisphere, the edges of which (*sa.e.*) are continuous with the large vacuolated cells. The concavity of the hemisphere is filled with large vacuolated cells (*l.v.c.*) which have separated from the edges of the saddle. All the nuclei belonging to the cells cut in the section are shown, although some are not situated in this section. The outer membrane is present, but only traces of the inner membrane could be detected, and these do not lie in this section.

FIGURE 104. Longitudinal section of the stage shown in figure 71, plate 37, for general description see legend to figure 71. The embryo has shrunk a little at fixation. The epithelial hemisphere now forms a continuous blastoderm over the large vacuolated cells. The latter are even more vacuolated, and tend to form a syncytial network (*l.v.c.*). No trace of the inner membrane remains. (Six nuclei of the large vacuolated cells have been inserted from neighbouring sections.)

PLATE 40

Sections of *P. balfouri*, magn. × 350 approx.

FIGURES 105 to 108 show transverse sections through the posterior thickening of the germinal disk at different ages.

FIGURE 105. Shows the earliest appearance of the posterior thickening, see figures 72 and 73, plate 37. It consists of the giant cell (*g.c.*) and about 14 undifferentiated cells (*u.c.*), four of which are internal, five undifferentiated cells are cut in the section, one being in mitosis, and one is internal. The germinal disk is sharply demarcated from the thin blastoderm (*t.bl.*) surrounding it, and the remains of the large vacuolated cells are still present (*n.*).

FIGURE 106. Shows the embryo reconstructed in figure 74, plate 37. The posterior thickening is larger. The cells surrounding the giant cell (*g.c.*) are more numerous; those situated internally are becoming vacuolated and forming an endodermal epithelium which extends internal to the giant cell and laterally beyond the germinal disk (*en.*) to the limits shown.

FIGURE 107. Shows an older stage intermediate between the embryos reconstructed in figures 74 and 75, plate 37. The giant cell has sunk below the surface, and the endomermal layer has extended. The remains of the large vacuolated cells are scanty. A few of the internal cells may be mesoderm, but they are not yet clearly differentiated.

FIGURE 108. Shows the embryo reconstructed in figure 75, plate 37, and seen in whole view in figure 76. The giant cell has sunk further from the surface, but is otherwise unaltered, only a small part of it is cut in this section. The endodermal epithelium has spread far beyond the figure, see figure 75. A blastoporal area (*b.a.*) is now differentiated from the surrounding blastoderm of the posterior thickening, and between the blastoderm and the endoderm lie the first mesodermal cells (*me.*). The remains of the large vacuolated cells are present and a degenerating nucleus (*d.n.v.*) is cut.

FIGURE 109. Transverse section of the same embryo as the last, see figures 75 and 76, plate 37, passing through the anterior thickening and the mouth-anus rudiment at the level indicated. The fixation of the much vacuolated and drawn out endoderm (*en.*) leaves the cell outlines fragmentary in places, as on the left of the lips. Ectoderm and endoderm are united at the lips (*l.*). The lips are joined by a thin sheet of protoplasm (*c.m.*). Degenerating remains of the large vacuolated (*d.n.v.*) are seen for the last time.

FIGURES 110 to 112. Show sagittal sections of embryos at successive stages after the shrinkage of the blastodermic vesicle has been completed. Mesoderm only is immigrating inwards from the blastoporal area.

FIGURE 110. Sagittal section of the embryo shown in figures 78 and 79, plate 38. The mouth-anus (*mo.-a.*) is widely open. The junction of ectoderm and endoderm at the lip edges is clearly seen. The endoderm is thicker and more vacuolated dorsally. The blastoporal area (*b.a.*) is the only point at which internal tissues merge into the surface layer. The mesodermal mass (*me.*) is compact, see figure 79.

FIGURE 111. Sagittal section of an embryo at the stage shown in figures 80 and 81, plate 38. Both ectoderm and endoderm are thicker than in the last section, and degenerating endoderm is piling up in the mid-dorsal region where clear degenerating nuclei (*d.n.*) are seen. The blastoporal area is larger and further removed from the posterior border of the mouth-anus. Mesoderm is more extensive, and the limit of the lateral bands is shown by the dotted line.

FIGURE 112. Sagittal section of the embryo shown in figures 90 and 91, plate 38. The mouth-anus has divided into mouth (*mo.*) and anus (*a.*), and the fused middle region of the mouth-anus lips has formed the thin ventral body-wall (*v.b.*), representing the ventral wall of the yolk sac. The thinner dorsal ectoderm on the left of the figure represents the transitory dorsal wall of the yolk sac (see figures 85 and 86, plate 38, of a younger stage in lateral view); elsewhere the ectoderm is thicker. The blastoporal area is larger, and the lateral mesoderm extending from the median mass shown (*me.*) is indicated by dotted lines, eight somites have separated. The genital rudiment (*g.*) is now differentiated from the inner part of the mesodermal mass. The thick mass of degenerating dorsal endoderm is larger, and the stomodoeal ectoderm is tucking in from the anterior border of the mouth (*st.*).

PLATE 41

FIGURES 113 to 118. Reconstructions of *P. capensis*, conventions as on plates 32, 34, and 38, for key see plate 34, and protrusion of lips of mouth-anus in figures 114 to 118 is indicated by dotted lines. Magn. × 100 approx.

FIGURE 113. Earliest stage obtained with open mouth-anus (*mo.-a.*), the beginning of immigration of mesoderm (*m.*) from the blastoporal area (*b.a.*), see figure 2*h* (p. 502), and the longitudinal section

of the same embryo shown in figure 123, and the older stage obtained by Sedgwick (figure 22, plate 31, 1885). About 26 mesodermal cells (grey) and the giant cell (*g.c.*) have become internal; the lips of the mouth-anus do not protrude.

FIGURE 114. The blasoporal area (hatched) has increased in size and become further removed from the enlarged mouth-anus. The mesodermal bands have grown forwards and show the rudiments of the cavities of the first pair of somites forming from one or two initial spaces. The giant cell has disappeared (at a stage very little older than that of figure 113), and germ cells are differentiated (*g.*). The lips of the mouth-anus now protrude (see figure 119).

FIGURE 115. Older stage, the narrowing of the mouth-anus opening is an artefact which occurred on fixation. The mouth-anus is longer and the mesodermal bands have spread further forwards. The initial spaces of several coelomic cavities have appeared, probably each representing the cavity of one somite. Genital rudiment present.

FIGURE 116. Older stage in which the mouth-anus is preparing to divide by fusion of the middle part of the lips. The mesodermal bands are irregular but more advanced. Several initial coelomic spaces have appeared, but these are more than the number of somites which will be formed from this part of the band. Genital rudiment present.

FIGURE 117. Older stage in which the mesoderm is further advanced, five pairs of somites being formed or indicated, but the mouth-anus shows no signs of constriction in the middle region. Genital rudiment present.

FIGURE 118. Older stage in which the mouth-anus has just divided, and the mesoderm is slightly more advanced. The thin regions between the germinal bands and the mouth and anus (*v.y.s.*) representing the ventral wall of the yolk sac have appeared. No genital rudiment could be detected, but the fixation is not perfect.

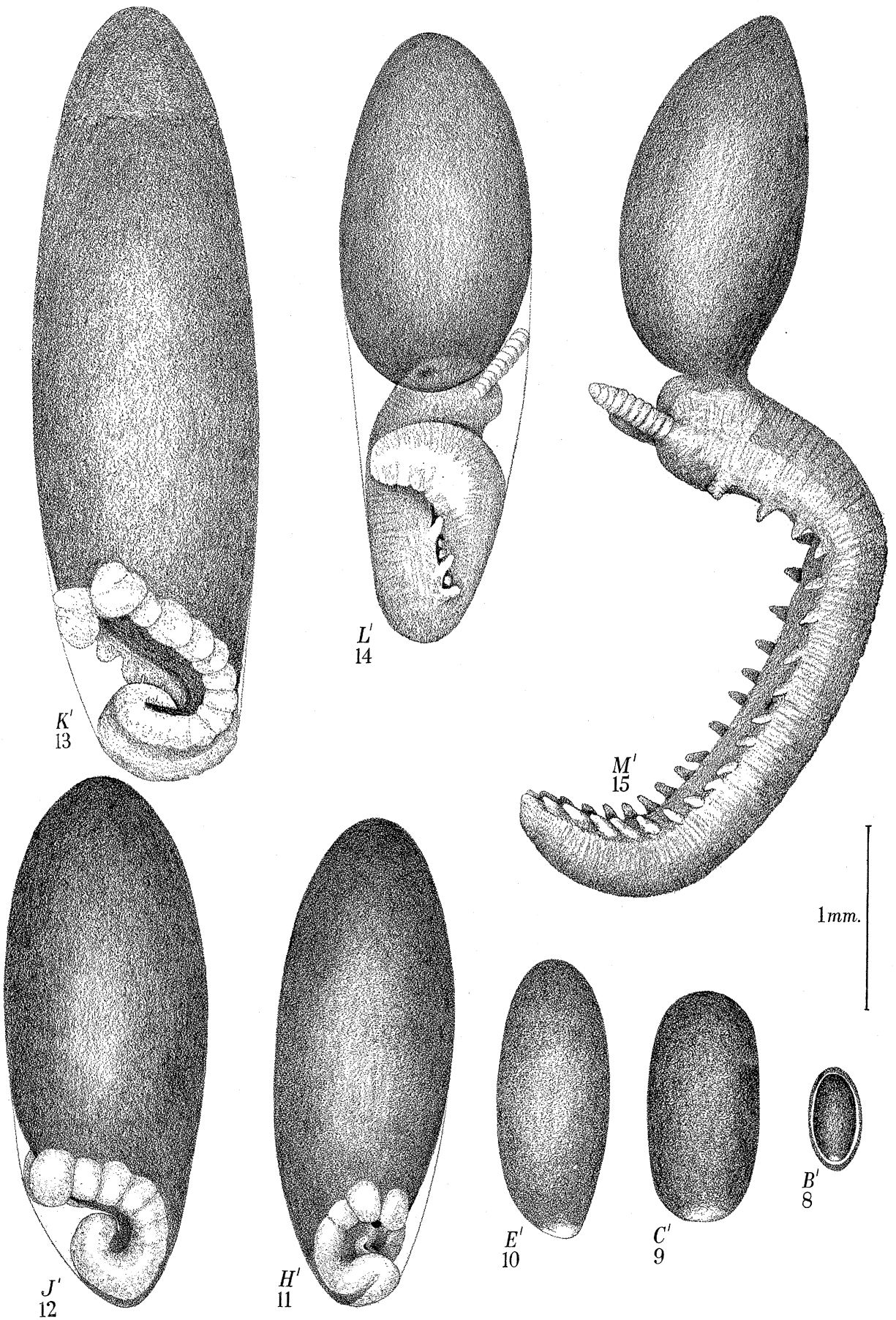
FIGURE 119. Transverse section of the stage shown in figure 116 at the level indicated, ectoderm white, endoderm roughly marked into cell limits, and mesoderm black.

FIGURE 120. Section across a late segmentation stage of about 80 cells, corresponding with stage *i*, plates 37 and 39 for *P. balfouri*. A disk or saddle of blastomeres (*sa.*) forms one side of the embryo and its concavity is loosely filled with large vacuolated cells (*en.*) destined to form the definitive endoderm. A few non-nucleated cytoplasmic spheres (*c.s.*) still remain. The inner parts of the blastomeres forming the saddle are vacuolated and the endodermal cells contain some lightly staining inclusions. The embryo lay within a very large membrane, see figure 2 (*m.d.*), p. 502. × 350 approx.

FIGURE 121. Section across the stage shown in figure 2*d*, p. 502. The disk of blastomeres has spread further round the large vacuolated endodermal cells, the latter form an irregular layer lining the gastrula, and some lie freely in the archenteron (*f.en.*) and partially plug the blastopore (*bp.*). × 350 approx.

FIGURE 122. Section across the stage shown in figure 2*f*, p. 502. The blastopore is reduced to a very narrow slit, and the archenteron contains fewer free endodermal cells. × 350 approx.

FIGURE 123. Longitudinal section of the embryo shown in figure 2*d*, p. 502 and in figure 113 at the level indicated. The section passes through the mouth-anus and the giant cell (*g.c.*), but it is not quite sagittal and so does not also show the middle of the blastoporal area (*b.a.*) or primitive streak from which mesoderm is immigrating. Four mesodermal cells lie in this section, the total number being about twenty-six. The giant cell contains secretory droplets of various sizes in its cytoplasm which is densely packed with mitochondria. Some of these droplets have passed out of the cell and lie elsewhere (*s.d.*). No free endodermal cells lie anywhere in the gut lumen. All the endodermal inclusions are now absorbed, other than the secretory droplets from the giant cell, and the inner parts of the ectodermal cells are still vacuolated. A transverse section through a similar stage showing the giant cell is shown in figure 3, p. 514. × 350 approx.



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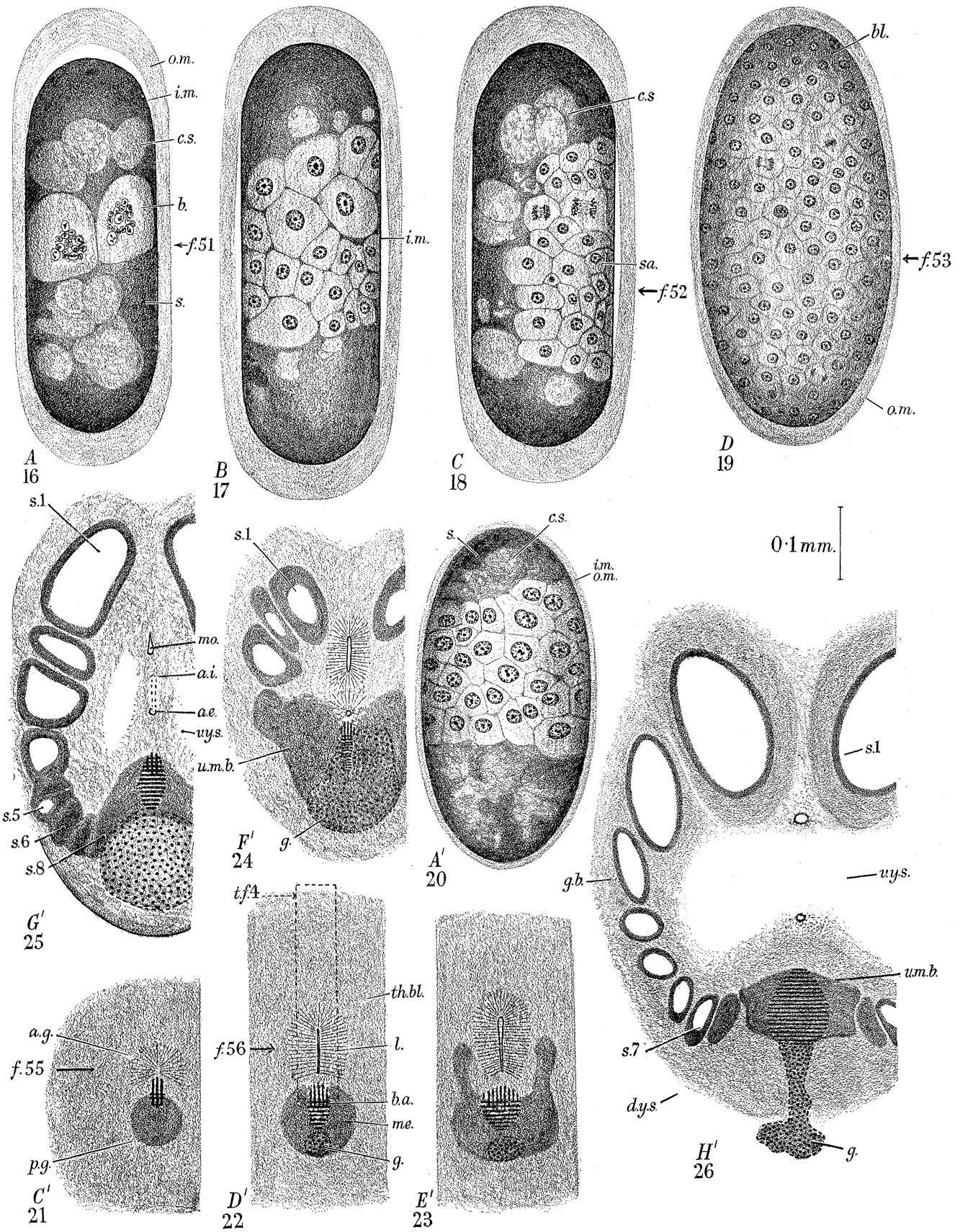
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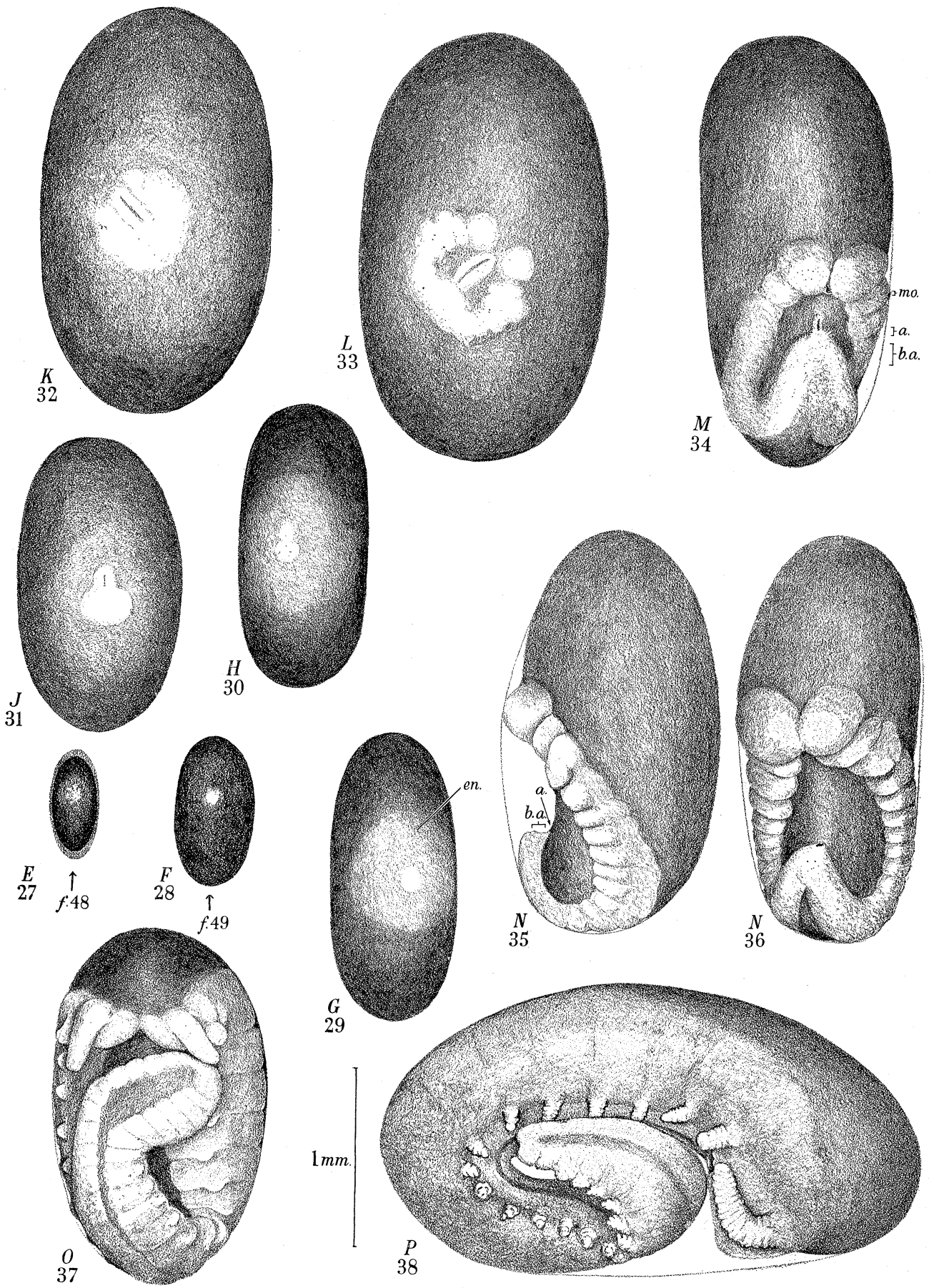
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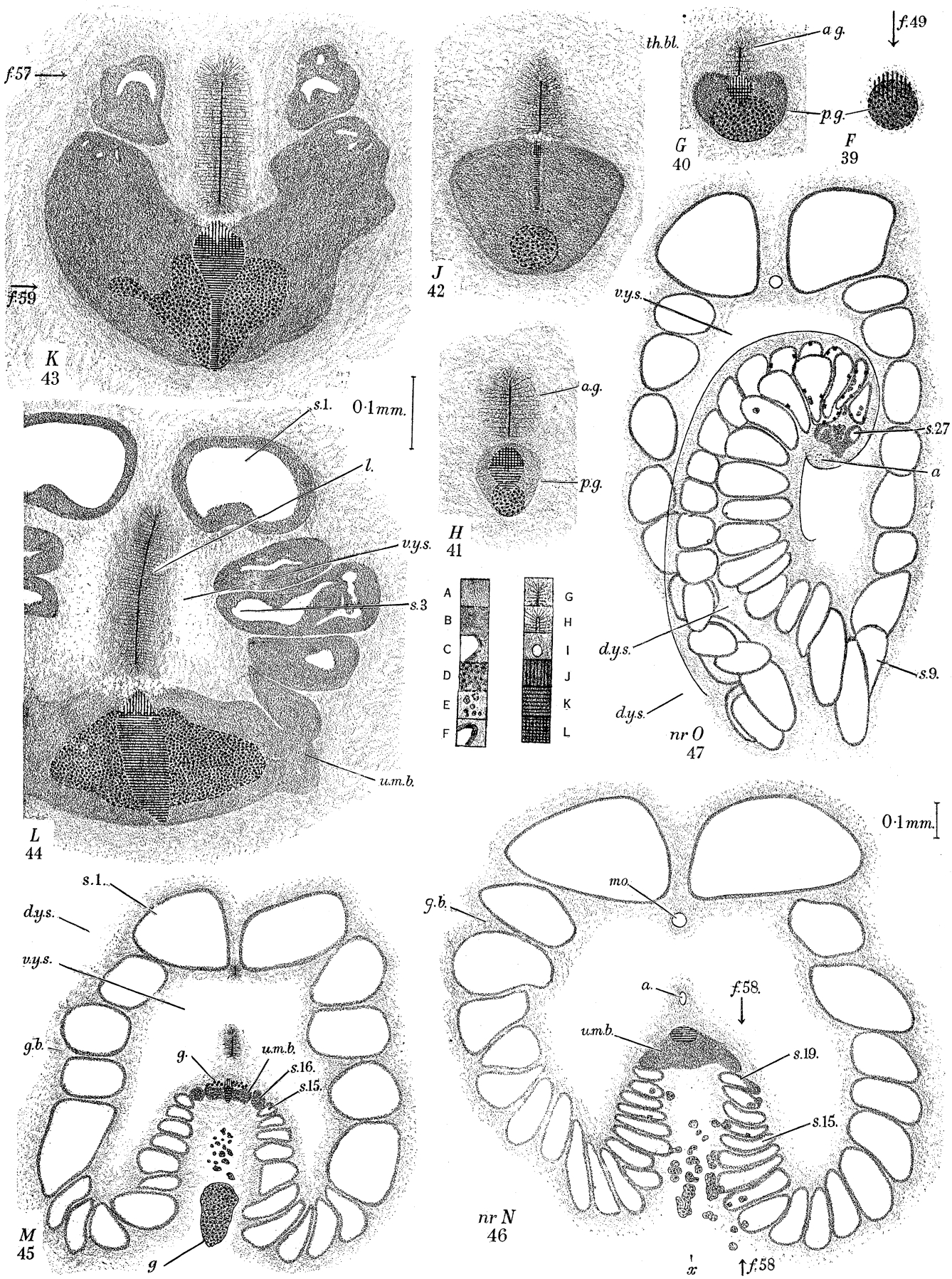
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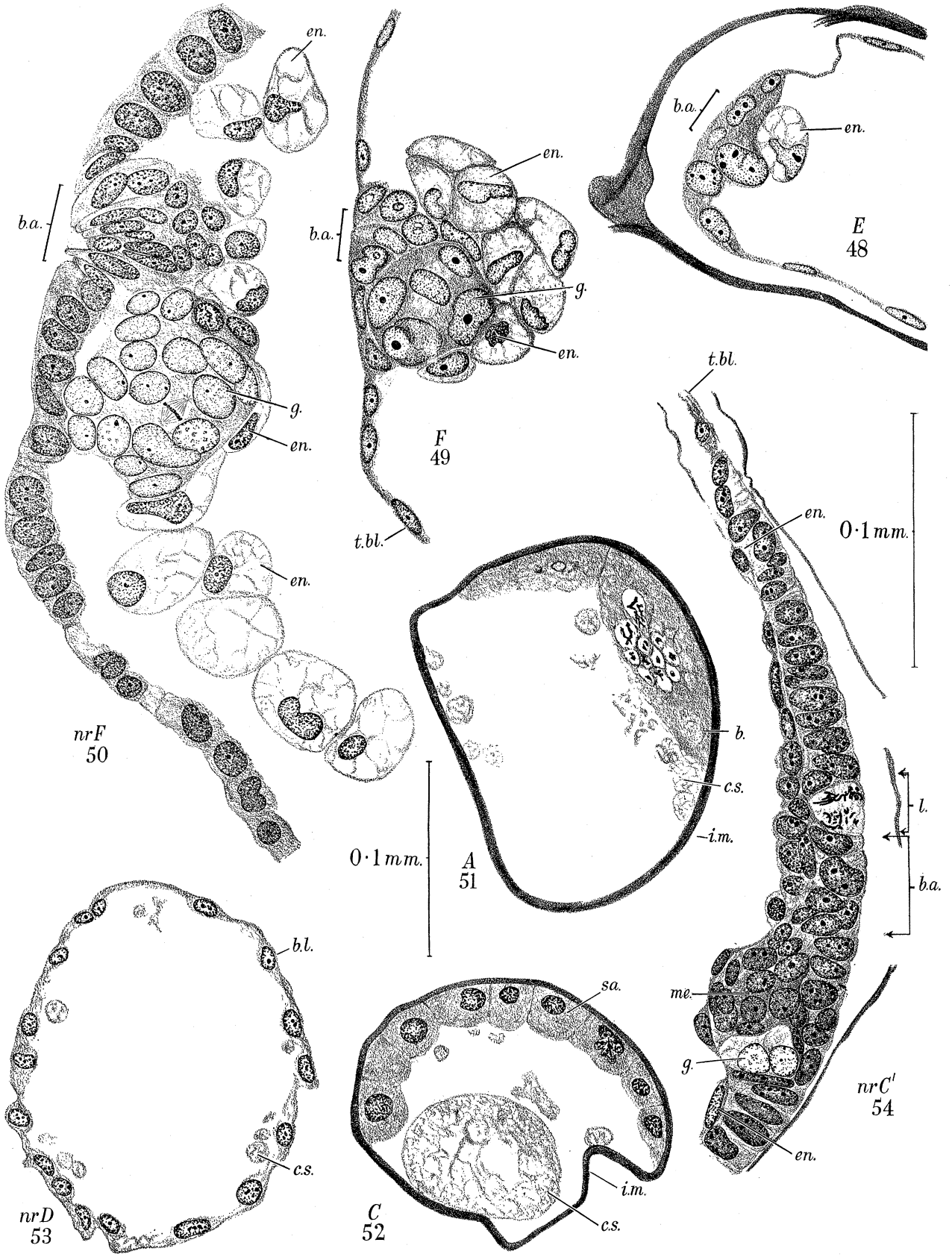
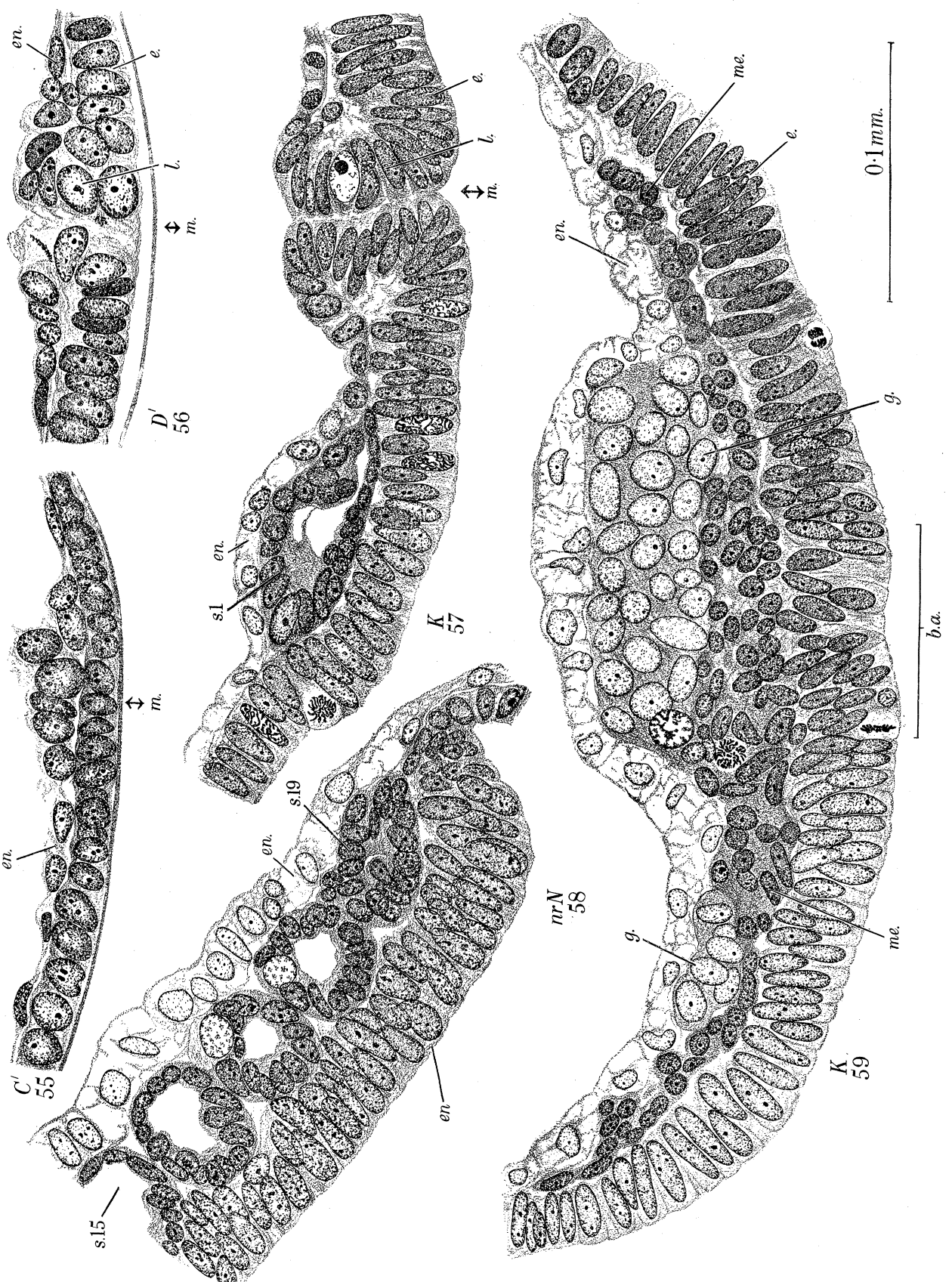
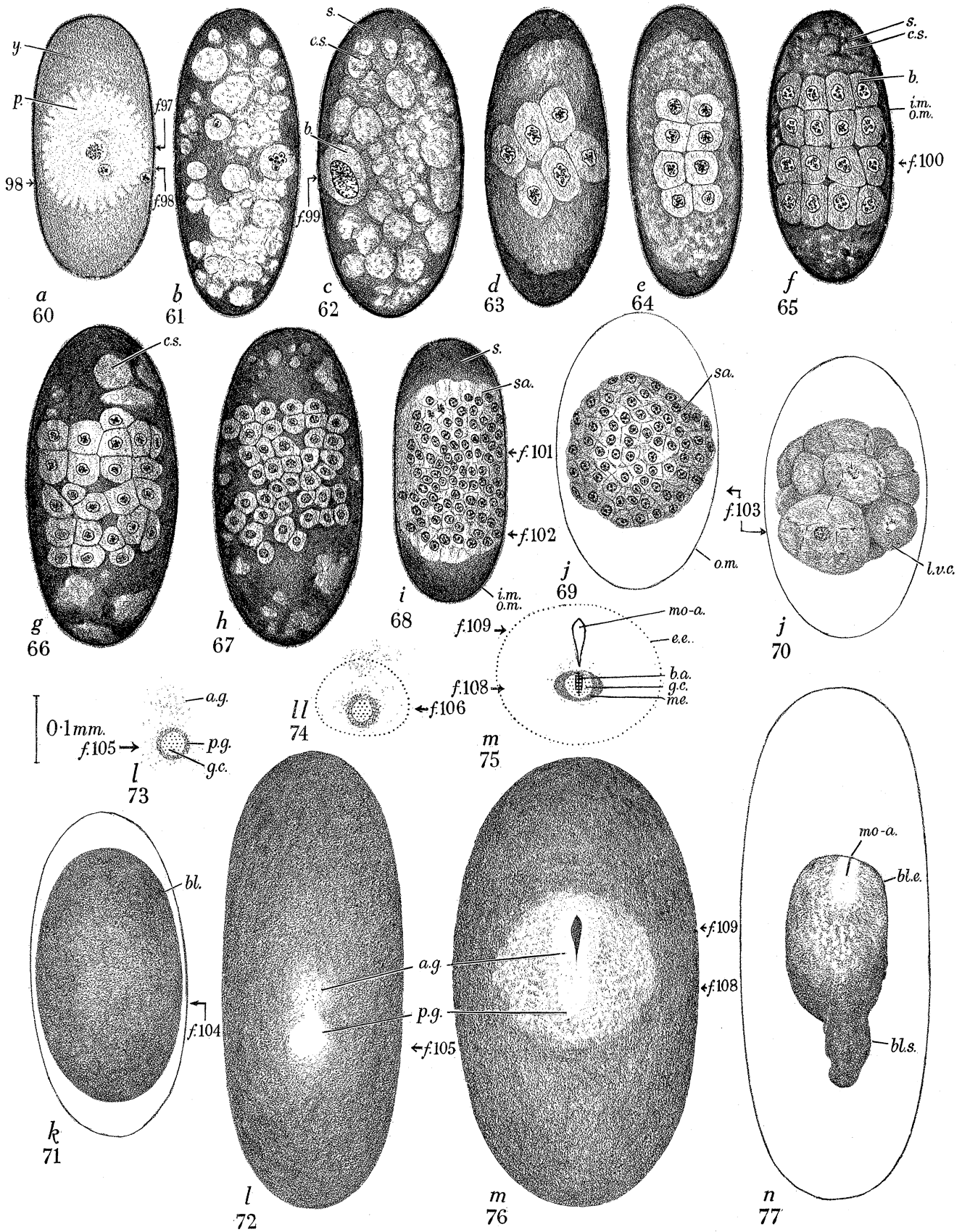
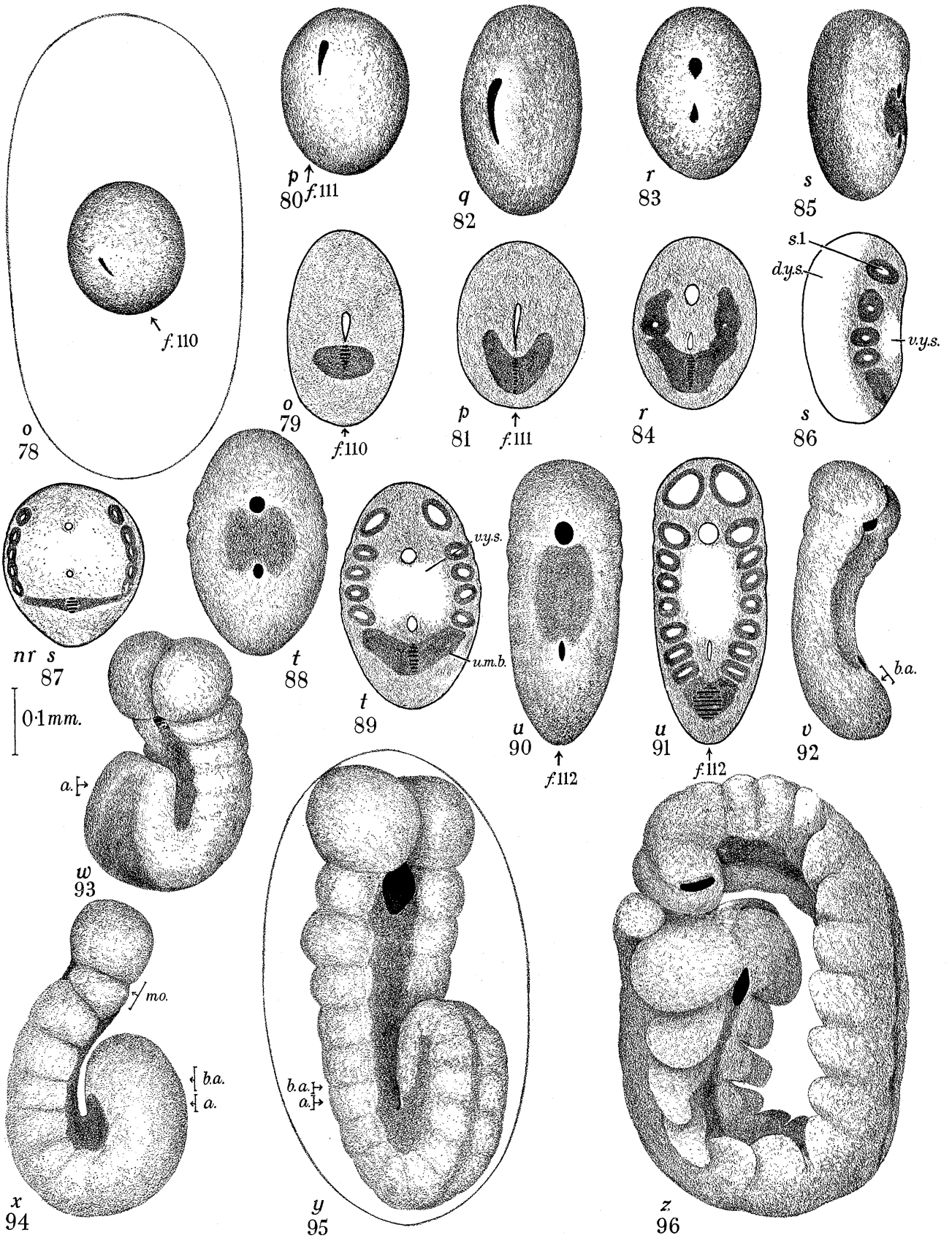
d.y.s.
s.9.
d.y.s.
nr O
47
0·1 mm.
s.1.
d.y.s.
v.y.s.
g.b.
g.
u.m.b.
s.16.
s.15.
g
M
45
mo.
g.b.
a.
f.58
u.m.b.
s.19.
s.15.
nr N
46
x
f.58

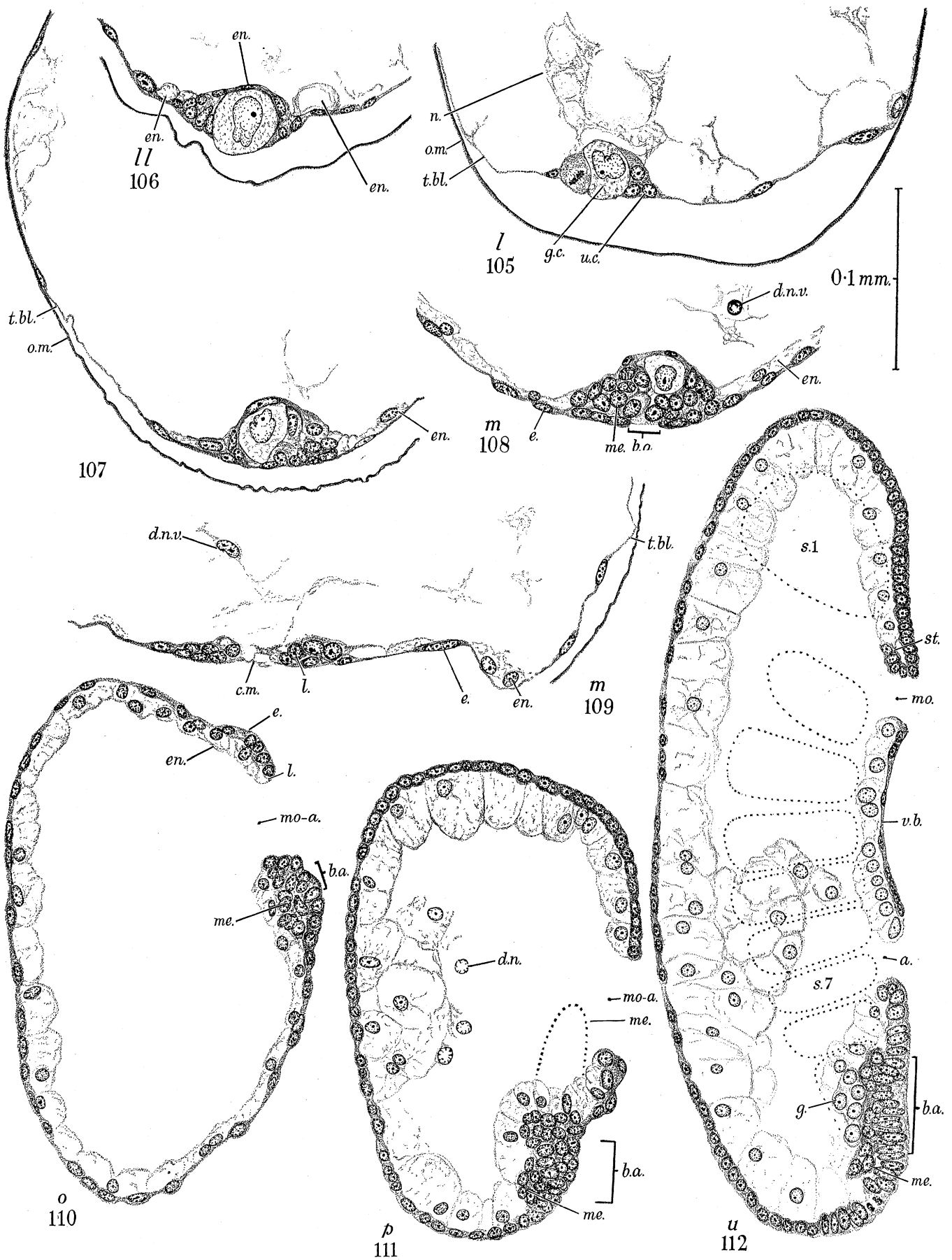

en.
b.a.
g.
en.
en.
nrF
50
b.l.
c.s.
nrD
53
b.a.
en.
g.
en.
t.bl.
F
49
0·1 mm.
b.
c.s.
i.m.
A
51
sa.
i.m.
c.s.
C
52
b.a.
en.
E
48
t.bl.
en.
0·1 mm.
l.
b.a.
me.
g.
en.
nrC'
54

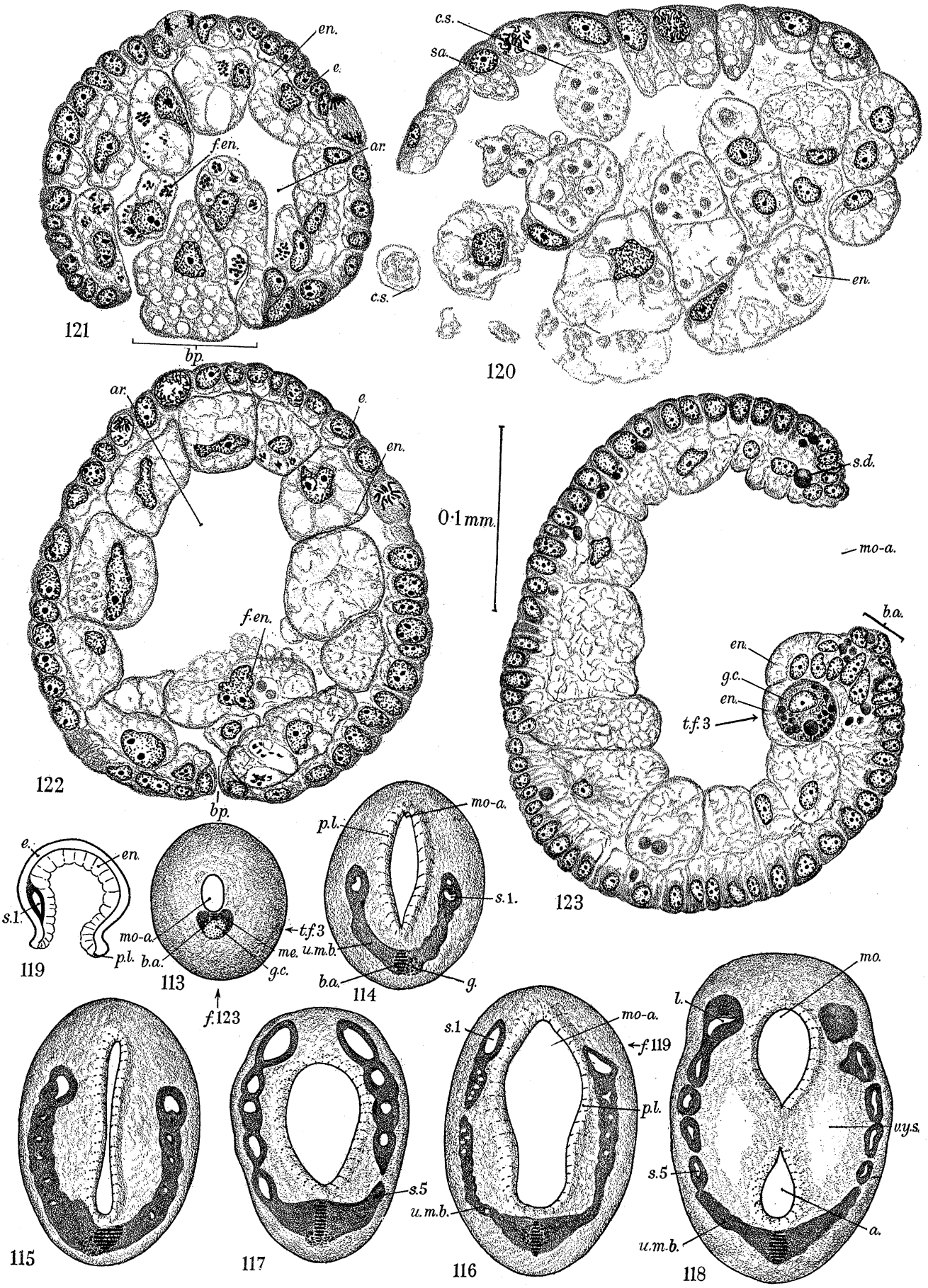

en.
e.
l.
m.
D′
56
C′
55
s.15
s.1
K
57
s.19
nrN
58
me.
g.
b.a.
K
59
0·1mm.


y.
p.
98
f.97
f.98
a
60
b
61
s.
c.s.
b.
f.99
c
62
d
63
e
64
s.
c.s.
b.
i.m.
o.m.
f.100
f
65
c.s.
g
66
h
67
s.
sa.
f.101
f.102
i.m.
o.m.
i
68
sa.
f.103
o.m.
j
69
l.v.c.
j
70
f.109
mo-a.
e.e.
b.a.
g.c.
me.
f.108
m
75
ll
74
f.106
0·1 mm.
f.105
a.g.
p.g.
g.c.
l
73
bl.
f.104
k
71
a.g.
p.g.
f.105
l
72
f.109
f.108
m
76
mo-a.
bl.e.
bl.s.
n
77


o
78
f. 110
p
80
f. 111
o
79
f. 110
q
82
p
81
f. 111
r
83
r
84
s
85
s.l.
d.y.s.
v.y.s.
s
86
nr s
87
t
88
v.y.s.
u.m.b.
t
89
u
90
f. 112
u
91
f. 112
b.a.
v
92
0·1 mm.
a.
w
93
m.o.
b.a.
a.
x
94
b.a.
a.
y
95
z
96

b
97
y.
i.m.
o.m.
p.
b
98
p.b.2
p.b.1
c
99
o.m.
i.m.
c.s.
f.
b.
f
100
o.m.
i.m.
c.s.
i.f.
b.
i
101
i.f.
sa.e.
sa.
c.s.
i.f.
sa.e.
i
102
f.
i.f
sa.e.
c.s.
l.v.c.
sa.
sa.e.
0·1 mm.
l.v.c.
B
1
sa.e.
j
103
l.v.c
o.m.
sa.
sa.e.
k
104
bl.
l.v.c.
bl.
l.v.c.
o.m.


en.
en.
en.
ll
106
n.
o.m.
t.bl.
g.c.
u.c.
l
105
0·1 mm.
t.bl.
o.m.
en.
107
d.n.v.
en.
m
108
e.
me.
b.a.
d.n.v.
t.bl.
c.m.
l.
e.
en.
m
109
s.1
st.
mo.
e.
en.
l.
mo-a.
b.a.
me.
v.b.
d.n.
mo-a.
me.
a.
s.7
g.
b.a.
b.a.
me.
me.
o
110
p
111
u
112


en.
e.
f.en.
ar.
121
bp.
c.s.
sa.
c.s.
en.
120
ar.
e.
en.
f.en.
122
bp.
0·1 mm.
s.d.
mo-a.
b.a.
en.
g.c.
en.
t.f.3
123
e.
en.
s.1.
119
mo-a.
p.l.
b.a.
t.f.3
me.
u.m.b.
g.c.
113
f.123
mo-a.
p.l.
s.1.
b.a.
g.
114
s.1
mo-a.
f.119
p.l.
s.5
u.m.b.
116
mo.
l.
u.y.s.
s.5
a.
u.m.b.
118
115
117