The Early Development of the Nemertean Cephalothrix rufifrons.

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With Plates 21–2 and 3 Text-figures.

CONTENTS

THE following account is based on material collected at Plymouth. Most of the embryonic and larval stages were obtained in the Spring and Summer of 1932 during which time I was holding the Student Probationership at the Marine Biological Laboratory. In April 1933 I was able to add most of the missing developmental stages.

The greater part of this research has been carried out in the Zoology Department of the University of Manchester, and to Professor H. Graham Cannon I owe my grateful thanks for his suggestions regarding technique and for helpful criticism during

the preparation of this paper. I am deeply indebted to Dr. E. J. Allen for assistance given during the early stages of this work.

Introduction and Historical.

Three distinct types of development are to be found among the nemerteans. Johannes Müller (1847) discovered, off the coast of Heligoland, a pelagic animal which he described as Pilidium gyrans. This he considered to be a larval form, and indeed showed, in 1854, that on metamorphosis it gave rise to a young nemertean.

Four years previously Desor (1850) had examined the larvae of Lineus ruber (O. F. Müller). Segmentation of the egg of this nemertean results in the formation of an embryo which rotates rapidly within the egg-membrane. The ciliated larval ectoderm is later thrown off, but not before a second, similarly ciliated layer—the definitive ectoderm of the adult—has been formed internally to the first. Lineus ruber is the only nemertean, in the development of which, Desor's larva is to be found, and the researches of Barrois (1877), Hubrecht (1886), Arnold (1898), and Nusbaum and Oxner (1913), among others, have made possible a detailed knowledge of the embryology of this form.

A number of authors including Metschnikoff (1870), Bütschli (1873), Coe (1899), C. B. Wilson (1900), and Salensky (1886 and 1912), have studied the development and metamorphosis of the pilidium; the accounts of the last three authorities are the more detailed. It is clear that great similarities are to be found in the development of the nemerteans through Desor's larva and through the pilidium, an observation not altogether surprising in view of the fact that both forms occur within the single genus Lineus, although the pilidium is also the larval form of nemerteans of other genera.

In both types of larva the adult ectoderm is of secondary origin, and is derived from five separate 'Anlagen', two antero-lateral 'Kopfscheiben', two postero-lateral 'Rumpfscheiben' and an unpaired postero-dorsal 'Rückenscheibe'.

A third, and in its simplest form probably the most primitive type of development, is also to be found. Here, the transition

from embryo to adult takes place without marked metamorphosis, and the embryonic ectoderm gives rise directly to that of the adult. In Cephalothrix galatheae (Dieck, 1874) and Emplectonema gracile (Delsman, 1915) the definitive ectoderm of the adult is formed within a provisional ectoderm which is later sloughed off, so that for a time the larva is enclosed within a ciliated sac. It seems probable that most nemerteans have a direct development of the type first described in Amphiporus lactifloreus (Barrois, 1877). Some fifteen species have since been shown to exhibit direct development, but only in six cases, namely, Prosorochmus viviparus Uljanin (Salensky, 1884), Prostoma vermiculus Quatrefages, and Drepanophorus spectabilis Quatrefages (Lebedinsky, 1897*a*), Geonemertes agricola Willemoes-Suhm (Coe, 1904), Malacobdella grossa O. F. Müller (Hammarsten, 1918) and Stichostemma graecense Böhmig (Reisinger, 1926) are descriptions given in any detail.

All these nemerteans are relatively complex forms. The nervous system is far removed from its primitive position in connexion with the ectoderm, and indeed lies in the parenchymatous tissue within the muscle layers of the body-wall. Furthermore, if the wide separation of the mouth and of the proboscis opening is to be regarded as a primitive character, then the Hoplonemertini and Bdellomorpha,[1] to which the above nemerteans belong, and which have the mouth near to, or actually in connexion with the proboscis through a common atrium, must be considered as advanced forms. There is little doubt that the Palaeonemertini (to which group the genus Cephalothrix belongs), with the nervous system situated within the muscles of the body-wall, with mouth and rhynchodaeum widely separated, and with other primitive features involving the ectoderm, nephridia and head-gland, more nearly approach the primitive stock. Of the development of this group practically nothing is known. Barrois (1877), McIntosh (1873), and Dawydoff (1928) have all noted that Cephalothrix linearis (Rathke) has a direct development,

[1] I have adopted the classification given by Böhmig (1929).

but give no details of the process. In the following description positive evidence concerning some of the more important features of development is lacking, but a general account of the embryology of this form is given in some detail.

Systematic Position of the Adult.

The simplicity of the adult structure of the nemerteans included in the genus Cephalothrix renders them admirable subjects for embryological study. Cephalic grooves and pits are absent and the head-gland is only slightly developed. Moreover, there is no secondary connexion of the oesophagus and rhynchodaeum and the ectoderm is of the simplest type. An excellent account of the adult anatomy has been given by Wijnhoff (1910).

There has been considerable confusion of the two species Cephalothrix linearis Rathke and Cephalothrix rufifrons Johnston. Bürger (1904) describes Cephalothrix linearis as being white often with a yellow tinge, as having no pigment spots and as being 100–150 mm. in length, and Cephalothrix rufifrons as being whitish with two small red-blue pigment spots at the extreme anterior end. The length in this case is 30–40 mm.

The specimens collected at Plymouth resemble very closely the Millport forms as described by Stephenson (1911). At the tip of the snout are two orange-red pigment spots. The distance between the brain and the mouth is rather more than three times the distance between the tip of the snout and the brain, and the length averages 75–100 mm. although isolated examples measure as much as 175 mm. On these characters alone it would be impossible to disagree with Stephenson who considers that there is no specific distinction, and that all the specimens must be included under the name Cephalothrix linearis Rathke, which has priority.

Wijnhoff (1913), however, has studied the question more closely, and although she regards the presence or absence of pigment as being specific, she has, from a study of the anatomy of the two forms, shown that there are also internal differences. The following are Wijnhoff's criteria of the two species.

Cephalothrix linearis Rathke. 'Farbe weiss oder mit geringem gelblichem Anfluge. Kopf etwas dunkler, aber ohne jegliche Augen oder Pigmentflecken. Länge 20 cm. Breite ± ½ mm. Die Gonaden finden sich lateralwärts von den Blutgefässen, welche sich an der ventralen Seite nähern. In der präoral region fehlt das Parenchym. Kopfdrüse vorhanden.'

Cephalothrix rufifrons Johnston. 'Äusserst dünne, farblose oder weissliche Art mit zwei kleinen roten oder blauroten Pigmentflecken an der Kopfspitze. Länge 30–40 mm. Breite 0·5 mm. In der präoral Region grosse Bindegewebeanhäufung; die Blutgefässe haben sich ventralwärts verlagert. Die Gonaden, welche sich medianwärts von den Blutgefässen finden, fangen erst in einiger Entfernung vom Ende des Vorderdarmes an. Kopfdrüse vorhanden.'

Because of the presence of parenchymatous tissue in the pre-oral region, and of the position of the gonads, which extend from the posterior end of the body to a point some distance behind the fore-gut in Plymouth specimens, I have adopted Wijnhoff's distinction and have referred my specimens to the species Cephalothrix rufifrons.

Material and Methods.

All the nemerteans collected for the purpose of obtaining eggs and developmental stages have been taken from the shore of Rum Bay, a small stretch of coast on the north-east margin of Plymouth Sound.

Males and females containing ripe sperm or ova were first found in the middle of March, and were obtained without difficulty until the end of August. Later than this the animals are hardly to be found at all, and on September 3, 1932 two hours collecting yielded only two specimens.

Collections were made at low water, and by far the greater number of animals were taken from the zone lying from 3–6 feet above datum. Cephalothrix rufifrons is found just below the surface in clean, fairly coarse sand, usually under stones. Under favourable conditions, an hour's collecting will yield 60–70 specimens, composed of males and females in approximately equal numbers.

Cephalothrix is very hardy and lives for an indefinite period under laboratory conditions. The usual procedure has been to keep 10–12 animals (males and females) in a large glass dish just covered with sea-water (ordinary tank-water such as circulates through the aquarium) changed each day. As they were kept in a cool place the temperature remained fairly constant. The females deposit their eggs and the males their sperm within 2–3 days of capture, and the strings of fertilized eggs, bound together by mucus, can be transferred to separate dishes and allowed to develop.[1]

All stages have been fixed in Bouin's fluid and in Bouin-Duboscq, and some few in Benoit's osmic fixative; the best results being obtained with warm Bouin-Duboscq. Embryos and larvae were washed in 70 per cent., and stored in 90 per cent. alcohol, Murray's open-tube pyroxylin method (Murray, 1924) being used, and were later embedded in clove-oil celloidin. Orientation of the later developmental stages can be effected without difficulty, but it has been found impossible to orientate the early embryos owing to the absence of obvious distinguishing features, so that whole masses of embryos, still retained within the common mucous envelope, have been cut at random.

Sections have been cut 6 μ thick and stained with Heidenhain's iron-alum haematoxylin, Delafield's haematoxylin and eosin, and with Mallory's triple stain. Iron haematoxylin is the only satisfactory stain for early stages, but Mallory's triple stain gives excellent results with older larvae.

Early stages of cleavage were followed in living eggs placed on a slide under a coverslip supported on plasticine feet. By this means the eggs and embryos can be rolled about and viewed from all angles. Good results have also been obtained by fixing, clearing, and storing in a mixture of equal parts of glacial acetic acid, glycerine, and sea-water, with subsequent tinging with acetocarmine as described by E. B. Wilson (1892).

External Changes During Development.

The general external changes which take place during the

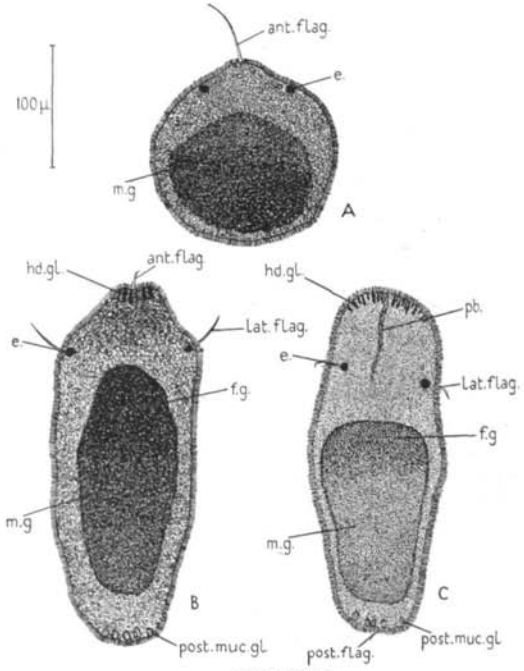
[1] The eggs are usually shed during the night. Only about 10 per cent. of the egg-strings were deposited during the hours of daylight.

development of Cephalothrix have been described by McIntosh (1873), and the following account, while adding to his observations, is also in agreement with them in all but a few details.

The eggs when laid are already fertilized (see p. 345) and are spherical in shape. Although the total diameter is about 130 μ, the actual diameter of the ovum is only about 105 μ in consequence of the thickness of the surrounding egg-membrane, within which two small polar bodies can be seen. Cleavage of the spiral type leads to the formation of a rounded blastula some 24 hours after fertilization. At 36 hours it is almost impossible externally to recognize the individual blastomeres, and the blastula has become depressed in the horizontal plane largely owing to flattening at the vegetative pole. Gastrulation is initiated at 45 hours as a result of invagination of the macromeres. The blastopore and larval mouth (see p. 349) wide at first, gradually close, until some 20 hours later there is no recognizable opening.

About 75–100 hours after fertilization, at a stage which sections subsequently show to correspond to the completion of gastrulation, the embryo begins slowly to revolve within the egg-membrane. Apart from the development of a ciliated ectoderm, no obvious differentiation of tissues can be seen either in the living embryo or in whole mounts after clearing.

Escape of the actively moving larva from the egg-membrane takes place 100–160 hours after fertilization. Larvae developed from ova fertilized on April 7, 1932, escaped 160 hours later, but during a very hot spell of weather in June of that year, 100 hours only elapsed between fertilization and hatching. Although a rise in temperature is without doubt responsible for an acceleration of development, other factors, such as yolk-content of the ovum, are contributory factors to the variation. Rupture of the membrane is effected mechanically by the 'nosing' movements of the young larva and is probably aided by a change in consistency of the jelly composing the egg-membrane. Some changes do take place, for the membrane surrounding the fertilized egg is much more sticky than at the time when the larva is about to escape.



Cephalothrix larvae drawn from life. A. Seven-day old larva (immediately after escape from the egg-membrane). B. Nineteen-day old larva. C. Thirty-three-day old larva. *ant.flag.*, anterior flagellum; *e.*, 'eye'; *f.g.*, fore-gut region; *hd.gl.*, head-gland; *lat.flag.*, lateral flagellum; *m.g.*, mid-gut region; *pb.*, proboscis rudiment; *post.flag.*, posterior flagellum; *post.muc.gl.*, posterior mucus gland.

At the time of hatching, the young ciliated larva is almost spherical and about 150 μ in diameter (Text-fig. 1 A). The anterior end tapers somewhat and bears a single long flagellum

(*ant.flag.*). Two 'eyes' (*e.*) consisting of an aggregation of pigment are placed laterally, a short distance from the anterior end. The whole larva is extremely opaque and the area of the invaginated mid-gut (*m.g.*) particularly so. Although sections show it to be present, the ectodermal stomodaeum cannot easily be distinguished in the living larva.

Immediately after its escape, the larva swims about with great rapidity, anterior end forwards, and all the time spinning slowly about its longitudinal axis. Larvae at this stage are positively heliotropic, and swarming towards the light remain suspended in the water as a dense cloud.

Some 12 days later (Text-fig. 1 B), the larva has elongated considerably and is slightly narrower (290 μ by 115 μ). Growth has taken place for the most part in the posterior direction, as can be seen by the changed position of the 'eyes' (*e.*) relative to the extremities of the body. In addition to the anterior flagellum (*ant.flag.*), which is now somewhat reduced in length, two lateral flagella (*lat.flag.*) have grown out on a level with the 'eyes'. The larva is slightly less opaque than formerly, and the gut is darkest in the anterior (stomodaeal) region (*f.g.*). At the tip of the head, darker areas mark the rudiments of the head-gland (Text-fig. 1 B, *hd.gl.*) while posteriorly a ring of large mucus secreting cells (*post.muc.gl.*) is clearly seen. The behaviour of the larvae has altered considerably, for no longer do they show any response, positive or negative, to the stimulus of light. They dart about indiscriminately, while some few sink to the bottom of the vessel to glide about in the manner of the adult, at the same time showing periodic wriggling and quivering movements. Many of them, while suspended in the water, bend themselves into the form of a U, the dorsal side forming the convex surface, the anterior and posterior tips of the body almost touching. A rapid somersaulting then takes place for a period of 5 seconds or so, the head end leading in the turn. The significance of this motion has not been analysed, but it cannot be associated with absorption of yolk, since all reserve food material has disappeared some time previously to this stage.

Later larvae (4–5 weeks after hatching) have lost the anterior

flagellum, while the lateral flagella (*lat.flag.*) are much shorter, but they have developed a short posterior flagellum (Text-fig. 1 c, *post.flag.*). The proboscis rudiment (*pb.*) can be seen faintly through the ectoderm, owing to the decreasing opacity of the tissues. The larvae are almost all to be found gliding about over the bottom of the vessel. When removed with a pipette they adhere to the glass at their posterior ends, no doubt as a result of the secretion of the large mucus glands (*post.muc.gl.*) situated there.

Larvae have been kept for a period of 8 weeks after fertilization, and by this time have diminished in size and have lost the posterior flagellum. They show no structural advance on those 5–6 weeks old, and the inability to rear them beyond this stage has been due to the failure to find suitable food.

The rudiments of all the adult organs, with the exception of those of the rectum and nephridia are, however, now well established.

Maturation, Fertilization and Formation of the Egg-membrane.

Male and female adult Cephalothrix, in captivity, gather together in a closely entwined, writhing mass, or lie side by side in an extended position; the mature males are white in colour and much thinner than the light brown females, which are so distended with eggs that they have the appearance of being segmented.

There is no duct leading from the gonad to the exterior, the eggs escaping merely by rupture of the body-wall. The eggs, when laid, are enclosed in a mucilaginous envelope secreted by ectodermal glands of the adult, and the spawn thus appears as a double row some 5 cm. long, each row representing the product of the gonads on one side of the animal. At the moment of laying, the membrane surrounding each egg is completely formed, the germinal vesicle has broken down, and an elevation on the surface of the egg marks the position where the polar bodies will shortly be nipped off.

When ova are removed from the female for the purpose of making an artificial fertilization, there is no membrane round

them, and the germinal vesicle is clearly marked as a lighter, eccentrically placed area of a diameter about one-fifth that of the egg (fig. 1 A, Pl. 21, *g.v.*). Each egg is, for the most part, very opaque, but around the inner opaque zone (*i.o.z.*) there is a peripheral, more pellucid zone (*o.p.z.*). After remaining for a short time in sea-water, the germinal vesicle breaks down, but no polar bodies are extruded. This stage corresponds to the formation of the first polar spindle and the eggs are now ready for fertilization.

Shortly after entrance of sperm into the ovum—whether more than one sperm enters the egg has not been determined—the surface of the egg begins to 'bubble'. The 'bubbles' coalesce, and become lifted off as a membrane, which about 1½ hours later, has attained its maximum thickness of 14 μ and constitutes the egg-membrane (fig. 1 B, Pl. 21, *f.m.*). The latter is, therefore, a fertilization membrane.

One can only conclude that in the ordinary course of events, fertilization is internal, and that even before the eggs are extruded they have passed through the maturation period and the egg (fertilization) membrane is formed. The interval between the times of institution of the membrane and the formation of the polar bodies (*p.b.*), represents the time taken for the sperm to pass from the surface of the egg to the female pronucleus. Internal fertilization is not uncommon among the nemerteans and has been recorded in many forms; Reisinger (1926) has actually seen the sperm within the ovary of Stichostemma graecense. The gelatinous egg-membrane is very elastic, and can be greatly deformed by pressure. Its surface is glutinous, and adheres to any object with which it happens to come into contact, but the glutinous character is lost to some extent as development proceeds, so that by the time the young larva is ready to escape from the membrane the original sticky nature has been lost almost entirely.

Cleavage and Formation of the Blastula.

Some 90–120 minutes after the polar bodies have been formed at a stage shown in fig. 1 B, Pl. 21, the first cleavage takes place, and this and all subsequent cleavages (at least, until the

thirty-two-cell stage) are completed in all the eggs of a single batch almost simultaneously.

The second, third, and fourth cleavages take place about 70, 140, and 185 minutes after the first, but these times are subject to variation.

Fig. 2 A, Pl. 21, is a median section through an egg just previous to the first division into two equal blastomeres. Yolk is distributed uniformly through the egg in the form of granules deeply staining with iron haematoxylin, and the egg is consequently very opaque; although the extreme peripheral zone (*o.p.z.*) is less so owing to the absence of yolk. The first place of cleavage passes through the point of extrusion of the polar bodies (*p.b.*) so that polarity is determined early, for the position of the polar bodies is dependent on the position of the germinal vesicle which in turn is formed while the egg is still within the body of the adult. The centrosomal areas are devoid of yolk, and the asters and nuclear spindle are well marked. It would appear that there are eleven or twelve chromosomes, but their small size makes it difficult to decide which of the two is the correct number.

The second cleavage is vertical and at right angles to the first —four apparently equal blastomeres resulting (fig. 2 B, Pl. 21). In the resting stage each nucleus (*nuc.*) is seen in section as a large clear area with a small nucleolus (*ncl.*). The cytoplasm, after fixation, has a finely granulated appearance, but large dark staining masses (*p.gran.*) appear round the periphery and remain until the initiation of gastrulation. Whether these masses, which appear in embryos fixed both in osmic and non-osmic fixatives, actually represent aggregations of yolk, or whether they are artifacts is doubtful, but they are useful in later stages in determining the boundaries of blastomeres which are otherwise not easily defined. At this stage the blastocoel (*bc.*) is well marked and the blastomeres are only in contact over a small portion of their surface.

The eight-cell stage follows as a result of the third cleavage, and is dexiotropic, but the degree of rotation of the first quartet of micromeres relative to the macromeres is variable; indeed, this cleavage in a minority of cases is almost orthoradial in

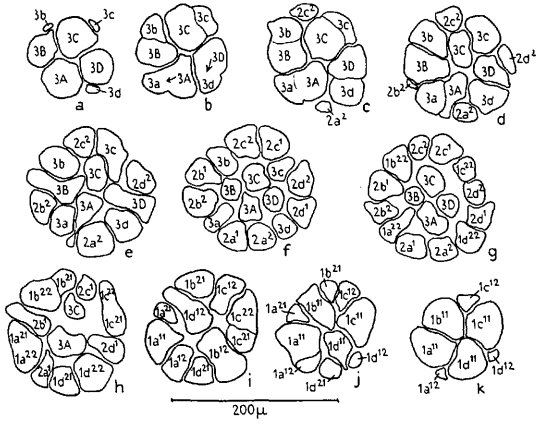
character. Whatever the degree of rotation of the first quartet, however, subsequent cleavages establish the spiral condition, and the thirty-two-cell stage results from further laeotropic and dexiotropic divisions of macromeres and micromeres. Twelve and twenty-four-cell stages have not been observed, and if formed can only be of a very temporary character. At the eight-cell stage, there is no external visible size distinction of the blastomeres, but already sections show that the macromeres project further than the micromeres into the blastocoel, and by the time the sixteen-cell stage is reached (fig. 3, Pl. 21) the four macromeres (*mac.*) are definitely the larger, their tips projecting far inwards into the segmentation cavity (*bc.*).[1] The blastomeres which are clearly marked off one from the other have their nuclei (*nuc.*) towards the outer part of the cell.

A series of horizontal sections through a blastula at the thirty-two-cell stage (Text-fig. 2) shows the disposition of the blastomeres. The four macromeres, 3 A–3 D project upwards almost to touch the micromeres of the first quartet. Externally (Text-fig. 2, *f.* and *g.*) the blastula presents a somewhat rectangular appearance owing to the jutting out of the micromeres $2a^1$–$2d^1$ and $2a^2$–$2d^2$ of the second quartet. A similar appearance has been noted in the blastulae of Malacobdella grossa (Hammarsten, 1918) and Stichostemma graecense (Reisinger, 1926).

Eighteen to twenty-four hours after fertilization, the blastula begins to show traces of horizontal elongation and the limits of the blastomeres can only be discerned with increasing difficulty. Fig. 4, Pl. 21, shows, however, that it is possible to define the cells at their periphery by means of the distribution of the peripheral granules (*p.gran.*) and by their nuclei, which begin to show an aggregation of chromatin into linin threads and have a well-marked nucleolus. At the close of blastulation—about 24 hours—the blastula is rather more than 100 μ long and about 80 μ high. The long axis will become the future antero-posterior axis of the larva while the vegetative pole marks the future ventral side.

[1] The macromeres themselves, however, are so similar in size that the designation A, B, C, D, can only be applied in a purely arbitrary manner.

According to Lebedinsky (1897) the 'Anlagen' of the adult organs are already distinguishable in the blastula, and many authors (see p. 355) have found primary mesoderm cells at this, or a slightly later stage. In Cephalothrix, at the close of blastulation, none of the 'Anlagen' of the adult organs are recognizable, neither are the primary mesoderm elements to be



TEXT-FIG. 2.

Outline drawings of successive horizontal sections through a thirty-two-cell stage blastula to show the disposition of the blastomeres. 3 A–3 D are the macromeres, while the designation of the micromeres follows the usual notation. Text-fig. 2, *a*, is through the vegetative pole, Text-fig. 2, *k*, through the animal pole. The egg-membrane is not shown.

found in the cells of the blastula wall. The four macromeres (*mac.*), numerous micromeres (*mic.*) and blastocoel (*bc.*) are still obvious, and it is not until gastrulation begins that cleavage becomes irregular. The macromeres then divide rapidly to produce the invaginated endoderm, and the mesoderm makes its appearance; the blastocoel, meanwhile, becomes completely, or almost completely obliterated.

Gastrulation Period.

For purposes of description, this phase includes all the developmental changes which take place from the beginning of invagination to the time when the embryo escapes from the egg-membrane to become a free-swimming larva. The mesoderm is formed during this period, but a description of its origin and proliferation is given under a separate heading (p. 345).

Invagination of the vegetative pole of the blastula begins about 24 hours after fertilization, and within the next 70–140 hours all the 'Anlagen', with the exception of those of the nephridia, head-gland and rectum are well established. The rapid proliferation of the mesoderm and the diffuseness of the gut, however, renders difficult the detection of the rudiments in their earliest stages. It is not until the gut has become re-organized by absorption of excess food material and the body-cavity[1] has appeared that it is possible to distinguish the 'Anlagen' with any degree of certainty. Nevertheless, the rudiments must be marked out even in the initial stages of gastrulation, for at 100 hours they are relatively advanced in development.

Gastrulation in Cephalothrix is effected by invagination, associated with rapid division of the macromeres. About this time the macro- and micromeres develop cilia, the movement of which results in the slow rotation of the embryo within the egg-membrane. Traces of cell limits are soon lost, and it is possible to define the gut in those forms in which yolk is present in any great amount only by the nuclei of the cells composing it.

As a result of invagination, a sac-like gut is formed with a lumen divided into two parts, at first (fig. 5 A and B, Pl. 21) in connexion through a narrower channel (*bp.*), but later separate (fig. 6, Pl. 21) owing to the closure of the channel. For some time there is no histological difference in the walls of the two sections of the gut, but later (at the time of escape of the larva) the walls of the outer part are seen to be of an ectodermal nature and to differ hardly at all from the general ectodermal cells of the body-wall. It is, therefore, clear that the narrow channel (*bp.*)

[1] The probable nature of the secondary body-cavity in Cephalothrix is discussed later in this paper).

referred to above represents the blastopore, and that the separation of the two parts of the gut corresponds to the closure of the blastopore and division of the gut into stomodaeum (*stom.*) (opening externally through the mouth) and mid-gut (*m.g.c.*). Invagination thus involves not only the endoderm which is to provide the mid-gut, but also the ectoderm of the larval and adult oesophagus.

This process is of particular interest because it is unique in the direct type of development and is, indeed, precisely similar to the condition found in the pilidium. Coe (1899) has shown that in the pilidia of Cerebratulus marginatus, Cerebratulus leidyi, and Micrura caeca the gut is divided into two portions, mid-gut and oesophagus, separated by a valve. He says 'The development of the intestinal tract in the pilidium, however, offers little positive evidence as to whether the oesophagus is ectodermal in origin, because in this form the whole digestive tract (except the rectum) is represented by an almost continuous invagination. From a comparison with other forms, as well as from the histological peculiarities of the oesophagus of the adult nemertean, it seems highly probable, as urged by Bürger in his splendid monograph, that this organ is here likewise of an ectodermic origin. A strong point in favour of this is the fact that the intestinal valve is indicated at a very early period. The histological differences likewise manifest themselves very early, . . . the process of invagination involves not only all of the entoderm cells on the lower pole of the blastula, but also some of the surrounding ectoderm cells, by a continuous process of infolding. The blastopore would be pushed inward and would be marked by the intestinal valve, or an homologous thickening of the epithelium.'

The sole difference between the Cephalothrix and pilidium condition would appear to be in the closure of the blastopore, for whereas in the pilidium the valve acts as a sphincter and is periodically opened to allow food to pass into the mid-gut, so that, strictly speaking, the blastopore never closes, in the gastrula of Cephalothrix the blastopore is completely closed although communication between the lumina of the fore- and mid-gut is later re-established by the formation of a channel at

the former point of closure (fig. 16, *ch.*, Pl. 22). The distinction is not fundamental and is clearly associated with the necessity in the free-swimming feeding pilidium of an opening into the gut, whereas in Cephalothrix the embryonic condition does not demand continuity of the lumina of fore- and mid-gut.

Considerable variation is found in the gastrulae as a result of the varying amount of yolk originally present in the egg. There was comparatively little yolk in the eggs collected in 1932, and in these forms the blastocoel (*bc*), although much reduced, is not completely obliterated until some hours after the beginning of gastrulation, as the section through a gastrula of 45 hours (fig. 5 A, Pl. 21) will show. The gut-cavity (*g.c.* and *stom.*), ciliated throughout, opens to the exterior by the widely open mouth (*m.*) and except for the region of the blastopore (*bp.*), midway along its length, has a broad lumen.

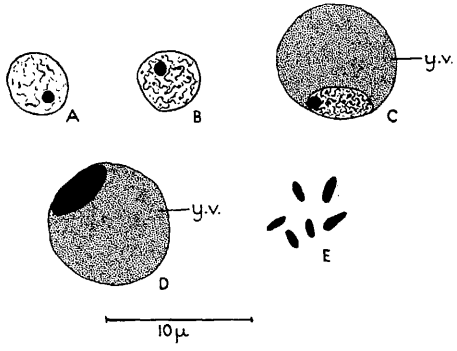
In the 1933 forms, however, the blastocoel is soon almost obliterated by proliferation of mesoderm (fig. 5 B, *mesod.*, Pl. 21) and expansion of the cells of the mid-gut (*m.g.*), and for a time previous to hatching, as this figure will show, it is impossible clearly to distinguish the germ-layers. Moreover, neither the mouth (*m.*) nor the lumen of the gut (*m.g.c.*) is as wide as in the previous state (fig. 5 A, Pl. 21).

During the gastrula phase there is to be seen a forward movement of the mouth. Partly owing to posterior elongation of the gastrula and partly to the forward growth of its posterior margin, the mouth (*m.*) is progressively constricted and is carried forward so that at 56 hours (fig. 5 B, *m.*, Pl. 21) it occupies an antero-ventral position. In consequence of this movement the gut (*m.g.*) projects upwards and backwards into the blastocoel.

Some time later—72 hours—the first sign of subdivision of the gut lumen appears in the narrowing of the lumen in the blastoporal region and before long, at 77 hours (fig. 6, Pl. 21), the blastopore (*bp.*) closes and the fore-gut (*stom.*) and mid-gut (*m.g.c.*) are separated. Now follows a period during which the limits of both the stomodaeum and mid-gut are difficult to make out, but gradually they become better defined, and further differentiation leads to distinction of a fore-gut obviously of an

ectodermal nature, and of a mid-gut in which the endoderm is of the adult type (fig. 11 A, Pl. 21).

At the beginning of the gastrulation period (fig. 5 B, Pl. 21) the cells of the gut-wall have the appearance of a broad syncytium, the outer margin of which merges imperceptibly into mesoderm (*mesod.*), and this in turn into ectoderm (*ect.*). Indeed, there is



TEXT-FIG. 3.

Successive stages of nuclear degeneration associated with yolk absorption. A. Normal nucleus with nucleolus and fine linin threads. B. Nucleus showing thickening of linin threads. C. Nucleus with aggregated chromatin and vacuole filled with yolk and other food material. D. The chromatin aggregated into a solid mass. E. The yolk and food material has been absorbed and the chromatin has been broken up into granules which are later digested. *y.v.*, yolk vacuole.

at this time no distinction of the germ-layers, and the blastocoel has disappeared. When the gut is established in its definitive form, however, the coelom has made its appearance. This change has been brought about by the rapid absorption of reserve food material by the cells of the definitive gut-wall associated with the degeneration of chromatin matter. The food material consists mainly of yolk, but it would seem probable, in view of the degeneration of chromatin, that whole cells are absorbed during the reorganization process.

In the cytoplasm of the gut, which in the earliest stages of gastrulation has a fine granular appearance, it is possible to distinguish at about 77 hours, or rather earlier, opaque areas of varying extent (fig. 6, *y.a.*, Pl. 21). Around the lumen of the gut, this darker appearance is particularly marked (fig. 8, *y.a.*, Pl. 21). Within the opaque areas are scattered masses staining deeply with iron haematoxylin, and there are found to be aggregations of chromatin derived from degenerating nuclei (figs. 6, 8, 7 A, 10, Pl. 21, fig. 15 A, Pl. 22, *deg.chr.*, *deg.nuc.*).

The absorption of yolk is associated with nuclear degeneration and comes about in the following manner. Here and there nuclei are to be found (Text-fig. 3 B) in which the threads are somewhat thicker than in the normal nuclei (Text-fig. 3 A). In the immediate neighbourhood of each of these nuclei is a vacuole within which yolk gathers, and it is these yolk-filled vacuoles (Text-fig. 3 C, *y.v.*) which constitute the opaque areas. The vacuole increases in size, and at the same time the nucleus undergoes progressive degeneration. The chromatin of the nuclear threads and of the nucleolus becomes aggregated into a solid mass (Text-fig. 3 D) which gradually breaks up into smaller and smaller pieces (Text-fig. 3 E). These latter are found for the most part around the lumen of the gut (figs. 6, 8, 10, Pl. 21, *deg.chr.*), and serve presumably, together with the yolk, as food for the rest of the gut cells. The extent of the mid-gut wall is diminished as the yolk disappears, the enteron becomes larger and the body-cavity appears (fig. 10, Pl. 21, *b.c.*). Although the absorptive process is mainly confined to the endoderm and ectoderm of the gut, the presence of isolated chromatin masses in the cells of the general ectoderm of the body-wall is indicative of a similar reorganization, though on a smaller scale, in this tissue.

Hammarsten (1918) describes a similar process in the cells of the mid-gut of Malacobdella, but here there is from the beginning a distinction between anteriorly placed 'Nahrungszellen' and the posterior definitive cells of the gut, the former serving as nourishment for the latter. Yolk absorption in Cephalothrix is of a more general nature in that the 'Nahrungszellen' are scattered among the other cells, and is

probably similar to the condition in Stichostemma where Reisinger (1926) describes the process briefly thus: 'Unter Einschmelzung von Zellmaterial höhlt sich die Entodermmasse zum Mitteldarm aus.'

While these changes have been taking place, and about 40–5 hours after fertilization, two symmetrically placed invaginations appear dorso-laterally, midway between the anterior and posterior ends of the gastrula (figs. 7 A, 7 B, Pl. 21, *ner.rud.*). The ciliated pits thus formed are associated with the first appearance of the rudiments of the future nervous system. Apart from the invagination of the gut at gastrulation, they are the most obvious of the 'Anlagen' appearing during development. The pits persist for about 24–30 hours, but then become cut off from the exterior, and in the 75-hour old gastrula their cavities have disappeared entirely.

Mesoderm.

In no feature of nemertean development has there been so much diversity of opinion as in the formation of the mesoderm. Differences of opinion have arisen, not only with regard to the number and position of the teloblasts, but also as to whether the mesoderm is budded off as dispersed elements into the blastocoel, or as definite bands of tissue. In this latter case the subsequent splitting of the bands into somatic and splanchnic layers might result in the formation of a true coelom, comparable to that of the annelids and molluscs. On the other hand, if the mesodermal cells exist as mere scattered elements in the blastocoel, then the body-cavity, when it appears later in development, might be regarded as a re-expanded blastocoel. Salensky (1909), Arnold (1898), Nusbaum and Oxner (1913), Coe (1899), Wilson (1900) and Dawydoff (1928) hold to the former view, while Hammarsten (1918) and Reisinger (1926), among others, believe that in the forms which they have studied there is no true coelom.

In the pilidium (Coe, 1899) and in Desor's larva (Nusbaum and Oxner, 1913) the mesoderm is said to arise from a single teloblast. Coe says 'it *appears* to arise from two somewhat indistinct, though closely connected sources, from a large pos-

terior pole-cell as in the annelids', and infers, therefore, that the teloblast in question is 4*d*. Nusbaum and Oxner claim on positive evidence, following a study of the cell lineage of the developing embryo of Lineus ruber, that it is indeed 4*d* from which the mesoderm is derived. In Emplectonema (Delsmann, 1915) and in Cephalothrix and Carinella (Dawydoff, 1928) there is no definite indication of the mesoderm deriving its origin from a single teloblast, and on this account Dawydoff (1928) considers that 'les recherches des auteurs polonais' (Nusbaum and Oxner) 'demandent une vérification soigneuse'.

Unless one can be perfectly certain of the origin and identity of the mesoderm during its early history, generalizations only make comparison with other forms unreliable, and for this reason the following account of the mesoderm in Cephalothrix will be found to be of a somewhat reserved nature.

In Cephalothrix the mesoderm is first apparent in the early gastrula (figs. 5A, 5B, Pl. 21, *mesod.*). Even at this early stage the blastocoel (*bc*) is only quite a small cavity owing to the deep invagination of endoderm cells. The mesoderm, however, divides rapidly and assists in the subsequent complete obliteration of the primary body-cavity. It would appear to arise from four teloblasts, situated in the angle of the blastocoel, antero- and postero-lateral to the invaginated endoderm. Fig. 9, Pl. 21, a parasagittal section through a gastrula of 36 hours shows two of the four primary groups of mesodermal elements (*nuc.mes.*). They are distinguishable only by their nuclei and it is impossible to find any trace of cell limits. The disposition of the four primary cells is similar to that found in Malacobdella (Hammarsten, 1918) where the teloblasts are represented by the blastomeres $2a^{1111}$–$2d^{1111}$. It has not been found possible, however, to determine by direct observation, the identity of the primary cells in Cephalothrix.

Throughout the whole of the later period of gastrulation, it is impossible in the Cephalothrix embryo to distinguish with certainty the limits of the three germ-layers. Figs. 5B, 6, 8, 7B, and 9, Pl. 21, will show that the nuclei are scattered in a seemingly indiscriminate manner, and the cytoplasm shows

not the slightest indication of cell limits. It is not possible, therefore, to say whether the mesoderm is primarily represented by scattered cells, which are later compacted together as a result of crowding in the blastocoel, or whether it exists from the moment of its origin as a solid band of tissue. If the former be the correct conclusion, then the body-cavity appearing after reorganization of the ectoderm and endoderm, is blastocoelic. On the other hand, the second condition would, on the splitting of the mesoderm into two separate layers, yield a true coelom.

In addition to the mesoderm, derived from the four teloblasts, mesenchymal elements are budded off from the ectoderm into the body-cavity. The nuclei of the mesenchyme (fig. 10, Pl. 21, *mesench.*) are very small and are first apparent as distinct elements during the time of reorganization of the ectoderm, and it is for this reason that it has not been possible to trace the precise origin of the mesenchyme. Subsequent to the stage figures it is impracticable to differentiate between mesoderm and mesenchyme.

The Late Embryo.

At the time of rupture of the egg-membrane, the larval gut-wall may already be almost devoid of yolk, and this was the case with the 1932 larvae, but in the 1933 forms, yolk is still in the process of absorption after 48 hours of free-swimming life (fig. 15 A, Pl. 22, *y.a.*). Generally speaking, however, the following description may be taken as typical of the organization of the embryo just before its escape from the membrane.

Fig. 10, Pl. 21, will show that the three germ-layers of the almost spherical embryo have yet to be organized into a condition where it is possible to distinguish the cell limits with ease. The ectoderm (*ect.*), externally ciliated, consists of irregularly shaped cell groups projecting into the cavity. Within the latter lie scattered mesoderm cells (*mesod.*) with diffuse cytoplasm, so that the only indications of their entity are their nuclei. The centrally situated mid-gut has a well defined almost circular cavity (*m.g.c.*), but ventrally the lumen is drawn out into a pocket (fig. 11 A, Pl. 21, *v.p.*) which is not shown in fig. 10,

Pl. 21, owing to the section being cut in an oblique horizontal plane. This ventral pocket is a relic of the more tubular portion of the gut, which, in earlier stages, opened into the fore-gut through the blastopore. The pocket is persistent and is found in the oldest larvae; into its base the stomodaeum later re-opens (fig. 16, Pl. 22, *v.p.*). At this stage, however, the ecto-dermal stomodaeum (fig. 10, Pl. 21, *stom.*) exists merely as a rather ill-defined pear-shaped invagination opening to the exterior through the pore-like mouth (*m.*).

Organization of the Young Larva.

The larva which has just escaped from the egg-membrane is equipped with almost all the rudiments of the adult organs. A description of the internal organization of the larva at this stage will serve as a basis from which it will be possible to follow the history of the various 'anlagen'.

The ectoderm is single layered, and consists of epithelial cells approximately triangular in shape, with attenuated apices projecting far into the coelom (figs. 11 A and B, Pl. 21, *epith.*). Externally each of the cells bears numerous cilia. The basal granules (*bas.gran.*), typical of ciliated cells, are prominent brown spots which do not stain with any of the reagents used. Within the delicately fibrillated cytoplasm is the nucleus—less prominent than that of the mesodermal and endodermal cell. Fixation and shrinkage cause the ectodermal elements to break away one from the other, but they are figured as they exist in the living larva.

In the mesoderm cell (*mesod.*) only the nucleus can be made out with certainty, although the more advanced of the muscular elements (*musc.el.*) are spindle-shaped as a result of elongation of the cytoplasmic portion.

The cytoplasm of the endoderm of the mid-gut (*m.g.*) already shows the fibrillar structure characteristic of the gut of the adult nemertean. Within the gut-cavity, and in the process of digestion, are usually to be found cells (*dig.*) rich in yolk and aggregated chromatin material—the sole remains of the substance absorbed and digested during the reorganization process. The cells of the endoderm are ciliated and the cilia project into the

lumen (*m.g.c.*). In the stomodaeal region this latter is elongated transversely (fig. 12 A, Pl. 21, *m.g.c.*) but more posteriorly, the plane of elongation is dorso-ventral (fig. 12 B, Pl. 21, *m.g.c.*).

The stomodaeum is represented as an ectodermal tubular invagination (figs. 11 A, 12 A, Pl. 21, *stom.*), the cells of its wall differing from those of the general ectoderm only in the fact that they bear longer cilia. Neither in the stomodaeum, nor in the general ectoderm, at this time, is there any differentiation of cells into glands.

A median sagittal section of the larva (fig. 11 A, Pl. 21) shows the gut (*m.g.*) to be roughly pear-shaped, with the pointed end projecting anteriorly. The stomodaeum (*stom.*) is directed upwards and backwards to meet the ventral pocket (*v.p.*) of the gut, the base of which represents the point of closure of the blastopore. There is, as yet, no connexion between the cavities of the mid-gut and stomodaeum, although the walls of the two are in contact.

Anteriorly to the stomodaeum, several of the nuclei lying in the body-cavity have their chromatin as a fine reticulum, dotted with fine granules, and are thus distinguished from the nuclei of the mesoderm cells in each of which there is a large nucleolus and a coarse reticulum. These nuclei are of ectodermal origin and belong to the nerve cells of the commissure (*v.comm.*) connecting the ventral ganglionic rudiments. Similar nuclei situated dorsally, and a little anterior to the first group are the corresponding elements of the dorsal commissure (*d.comm.*).

Directly anterior to the gut, between the dorsal and ventral commissures, are a few elongated cells (*prb.*). These are ectodermal cells which have migrated into the body-cavity and are the rudiments of the proboscis.

The ganglionic 'Anlagen' are seen to advantage in a parasagittal section lateral to the stomodaeum (fig. 11 B, Pl. 21). The 'Anlagen' of the dorsal ganglia (*d.g.*) lie just behind the 'eyes' (*e.*)—aggregations of large, brown, non-staining granules, while the ventral ganglia rudiments (*v.g.*) are more posteriorly situated. Between the two masses, scattered nerve nuclei represent the progenitors of the lateral commissures (*l.comm.*). Extending back from the ventral ganglia, a row of nerve nuclei marks the course

of the future lateral nerve-cords (*l.n.c.*), which bend slightly upwards as they reach the mid-gut region and are here found (figs. 12A, 12B, Pl. 21, *l.n.c.*) in the body-cavity lateral and slightly ventral to the gut.

Fig. 12A, Pl. 21, a transverse section through the stomodaeum, cuts across the lateral cords (*l.n.c.*) just behind the ganglia. Around the base of the stomodaeum are numerous nerve nuclei (*stom.n.*), the forerunners of the stomodaeal nerve, and it would appear (p. 369) that they arise by immigration from the ectoderm of this region. The relations of the dorsal and ventral ganglia and of the commissures are better seen in fig. 13, Pl. 22. Even at this early stage the neuropilem of the ganglia and commissures (*neurop.d.comm.*, *neurop.v.comm.*, *neurop.l.comm.*) is well developed as a finely fibrillated mass. The neuropilem of the ventral commissure (*v.comm.*) exists as a broad band, while that of the dorsal and lateral commissures is rather less extensive.

'Anlagen', which do not appear until the larva has developed beyond the condition described above, are those of the rectum, head-gland and nephridia. The origin and development of the various rudiments and tissues may now, with convenience, be separately described.

Differentiation of the Organ Rudiments.

Ectoderm.

From the epithelial cells of the general body ectoderm there are differentiated, during the course of development, two types of gland. By far the most conspicuous are the large mucus glands opening directly to the exterior, and confined to the posterior end of the body (figs. 14, 16, 20B, 21, Pl. 22, *post.muc.gl.*).

The living larvae are difficult to detach from surfaces over which they move. They adhere by their posterior ends only, and adhesion is maintained by the mucous secretion of the above-mentioned glands. The secretion would appear to be of a very viscous nature, for sections of larvae in which the fluid is partly exuded, show that on reaching the exterior it expands into a large globule (fig. 14, Pl. 22, *post.muc.gl'.*). In the normal condition each gland is in the form of an ellipsoid about 15 μ by 8 μ, so that a part of its bulk lies below the level of the epithelium.

The contents stain very readily with eosin, acid fuchsin and iron haematoxylin. These glands do not occur in the adult Cephalothrix.

The only other glands to be found in the ectoderm are small goblet-shaped cells (figs. 16, 17 A, 18, 19, 20 A, 21, Pl. 22, *ect.gl.*) which would appear to correspond to the 'Becherzellen' (Wijnhoff, 1910) of the adult. The interior of the goblet cell is sometimes granular (*ect.gl.*) in nature, but on occasions the gland is filled with fluid (figs. 17, 18, 19, Pl. 22, *ect.gl'.*). These differences no doubt correspond to different phases of secretory activity of the gland, but under all conditions the contents stain with eosin and acid fuchsin but not with the haematoxylins.

The anterior, lateral, and posterior flagella of the Cephalothrix larva are of ectodermal origin. Each flagellum is in the form of a brown, twisted, non-staining fibre which, as well as projecting outwards from the surface of the body, extends some distance into the interior of the larva (fig. 15 B, Pl. 22, *lat.flag.*). The lateral flagella thus come into contact with nervous elements, and it is conceivable that they are of a sensory nature. In the region of the mouth (fig. 18, Pl. 22, *flag.stom.*) are smaller flagella of a similar type; internally they are in contact with the stomodaeal nerve-ring, and this perhaps represents a mechanism for sensing the presence of food in the region of the mouth.

As far as the development has been followed, the formation of a basement membrane beneath the epithelial layer of the ectoderm has not been observed.

Stomodaeum.

The embryology of the nemerteans is remarkable for the number of different ways in which the fore-gut of the adult may arise. With the exception of Hubrecht (1886) and Salensky (1884, 1909) all workers agree that it is of ectodermal origin. Arnold (1898) and later Nusbaum and Oxner (1913) have shown that Hubrecht was in error in considering the fore-gut of the Desor larva of Lineus ruber to be of endodermal origin, and it is difficult to accept Salensky's findings in Prosorhochmus vivipara in view of the general ectodermal nature of the invagination.

In the pilidium, at gastrulation, a continuous invagination results in the blastopore being carried into the enteron to furnish the communicating channel between the endodermal mid-gut and the ectodermal oesophagus.

Gastrulation, in Desor's larva, involves the endoderm only, but nevertheless the blastopore is carried inwards in the same way. The ectodermal oesophagus is derived from two lateral diverticula which absorb the cells of the primary fore-gut and eventually connect up with the mid-gut (Nusbaum and Oxner, 1913).

There has been general agreement (Salensky excepted) that in nemerteans with direct development the stomodaeum arises as an ectodermal invagination in the region of the blastopore which, however, does not migrate inwards. In Drepanophorus (Lebedinsky, 1897*a*), Malacobdella (Hammarsten, 1918) and Stichostemma (Reisinger, 1926) the primary stomodaeum closes, and the mouth is formed secondarily in connexion with the proboscis—both oesophagus and rhynchodaeum opening into a common atrium.

The situation, in Cephalothrix, is different from that occurring in any other nemertean in which the direct development has been described. It has been shown that the stomodaeum arises as in the pilidium, and similarly provides the fore-gut of the adult. Only in the closure of the blastopore, in Cephalothrix, is there any difference between the two forms, and this is more apparent than real, for communication between fore- and mid-gut is re-established at the former point of closure. Discussion of this and other relevant points is reserved until later in this paper, and meanwhile the further development of the stomodaeum, after the closure of the blastopore, is described below.

At the time of rupture of the egg-membrane, the stomodaeum already shows some traces of its later differentiation. Its cells develop cilia which project into the somewhat ill-defined lumen (fig. 10, Pl. 21, *stom.*). The walls of the stomodaeum merge into those of the mid-gut in the former position of the blastopore (*bp'.*). The next stage is illustrated in fig. 15 A, Pl. 22, a transverse section through the stomodaeum of a larva 2 days after escape from the egg-membrane—one of the more slowly developing

1933 forms. The lumen of the stomodaeum (*stom.*) is clearly marked and is wider internally than at the mouth. Although the cells of the fore-gut are ciliated, there is no trace of basal granules, even though these are present in the cells of the general ectoderm (*bas.gran.*). Basal granules soon appear, however, and the stomodaeal cells become similar in all respects to those of the rest of the ectoderm—triangular in shape with their apices projecting into the coelom. The lumen becomes tubular and projects upwards and backwards from the mouth towards the ventral pocket of the mid-gut (figs. 11 A, 12 A, Pl. 21, *stom.*). Participation in the general antero-posterior lengthening of the larva results in the stomodaeum becoming slit-shaped (figs. 16, 18, Pl. 22, *stom.*) and after some 5 days of free-swimming life, the stomodaeal and mid-gut walls thin out, and are eventually perforated at the base of the ventral pocket (fig. 16, Pl. 22, *v.p.ch.*) so that oesophagus and mid-gut are in communication.

Meanwhile unicellular glands have appeared in the stomodaeal wall (fig. 18, Pl. 22, *stom.gl.*). They are longer than the ordinary epithelial cells and are particularly numerous around the upper part of the lumen. Their finely granular contents stain deeply with acid fuchsin and eosin, but not with mucicarmine. These glands are similar to the 'Körnchendrüsenzellen' described by Wijnhoff (1910) in the adult fore-gut, but mucus cells ('Schleimdrüsenzellen') are absent.

Mid-gut.

The mid-gut of nemerteans is always of an endodermal nature, resulting either from invagination or 'Einwanderung' (Hammarsten, 1918) of cells during gastrulation. Invagination of the simplest type is found in the pilidium where there is little reserve of yolk, and the mid-gut is well defined from the time of its initiation. The relative absence of yolk is, in this form, correlated with the early assumption of an active larval life and commencement of feeding.

In Desor's larva, and in nemerteans with direct development there is, after gastrulation, reorganization of the mid-gut cells associated with yolk absorption. Lebedinsky (1897*b*) does not specifically mention this reorganization in Drepanophorus

and Prostoma, but his summary of the course of events leads one to conclude that some such process does occur, although Bürger (1897) considers that in Drepanophorus all the original endoderm cells contribute to the wall of the mid-gut. Lebedinsky (1897*a*, p. 548) says, 'Später wird die Darmwand mehrschichtig und die Gastralhöhle verkleinert sich stark. Die Mehrschichtigkeit der Darmwand ist durch die Quertheilung der Entodermzellen verursacht; dieselbe ist vorläufig, in den spätesten Stadien bekommt die Darmwand ihren anfänglichen Charakter wieder, indem sie aus einreihigem Epithel besteht und eine röhrenförmige Gastralhöhle begrenzt.' Hammarsten (1918) describes the process in detail in Malacobdella, and Reisinger (1926) makes mention of it in Stichostemma. It seems probable that the condition is fairly general in nemerteans with direct development.

The fate of the mid-gut of Cephalothrix has been followed up to the time of establishment of a single-layered wall. In older larvae the mid-gut completely hides the stomodaeum dorsally (fig. 18, Pl. 22, *m.g.*), and behind the latter the ventral pocket bulges out laterally (fig. 19, Pl. 22, *v.p.*). As a result of this lateral expansion, the lateral nerve-cords would normally diverge in their passage to the hinder end of the body, but the divergence is minimized by the development of a shallow longitudinal groove in the walls of the mid-gut, within which the cords come to lie (fig. 19, Pl. 22, *l.n.c.*). The indentation of the outer wall is reflected in the shape of the gut-lumen into which two well marked lateral ridges project (fig. 19, Pl. 22, *lat.ridg.*). In addition (fig. 20 A, Pl. 22), a dorsal (*d.ridg.*) and a ventral ridge (*v.ridg*) is developed just behind the ventral pocket so that in this region the lumen has four horns extending dorso- and ventro-laterally (*d.l.h.*, *v.l.h.*). At the very end of the mid-gut these disappear, and the lumen has the form of a laterally expanded slit.

The glands of the mid-gut (figs. 16, 19, 20 A, Pl. 22, *m.g.gl.*) are of a similar type to those of the fore-gut and have the same staining reactions. In the anterior part of the gut the glands are numerous, but posteriorly they are not present in any great number. This condition persists in the adult; Wijnhoff (1910)

says, 'An einigen Exemplaren sowohl von Cephalothrix filiformis wie rufifrons ist das vordere Ende des Mitteldarmes von diesen Drüsenzellen ganz erfüllt; weiter hinten treten aber immer mehr Epithelfadenzellen auf, bis wie auch im sogennanten Enddarm, alle Drüsenzellen verschwunden sind'.

'Hind-gut'.

Among those nemerteans in which the direct type of development has hitherto been described, the hind-gut arises as an ectodermal invagination, which in Prostoma and Drepanophorus appears relatively early—'gleichzeitig mit der Rüsselanlage' (Lebedinsky, 1897 *a*). The proctodaeum 'Anlage' is marked out in the embryo of Malacobdella (Hammarsten, 1918) at 76 hours, while in Stichostemma, Reisinger (1926) merely states, 'Sehr spät bildet sich der Anus'. A similar late development of the ectodermal hind-gut has been noted in Desor's larva (Nusbaum and Oxner, 1913). Salensky (1884, 1909) failed to find a proctodaeal 'Anlage' in Prosorhochmus (Monopora) and on this account was subjected to criticism by Lebedinsky (1897 *b*) who says, 'Es ist unwahrscheinlich dass ein Monopora-Embryo, bei welchem der Oesophagus mit dem Atrium und mit dem Darm schon communicirt, und dieser letzte die Darmtaschen schon hat, keinen After besitze! Der "Embryo" von Monopora, den Salensky in Fig. 42 abbildet und ihm das Rectum absagt, ist wenigstens 10 Tage alt: derselbe stellt eine ganz fertige Nemertine dar.'

This criticism is thoroughly unsound, for in the pilidium we have an excellent example of a larva in which the gut has a single opening—the stomodaeum—throughout its life, and food both enters and leaves by this aperture. Indeed, it is not known how the hind-gut arises in the nemertean developed through the pilidium.

I can state confidently, that there is no trace of a proctodaeum in the larva of Cephalothrix, even when it is 6 weeks old. Wijnhoff (1910), as a result of histological examination of the nemertean gut, is of the opinion that a true hind-gut, as distinct from the mid-gut, is not to be found in Cephalothrix. There is no histological distinction between the mid-

gut and the so-called hind-gut, whereas the fore-gut (of ectodermal origin) is sharply defined, histologically.

I am strongly inclined to the view that the hind-gut in Cephalothrix, and probably also in the pilidium, is formed as a result of backward growth of the gut, with subsequent perforation at the posterior dorsal wall of the larva to form the anus, and is not formed as an ectodermal invagination. This, on positive histological and circumstantial embryological evidence, would seem to be a reasonable point of view. The ectodermal rectum of the more specialized nemerteans with a direct development I believe results from a telescoping of developmental processes, so that an ectodermal invagination grows in to meet the endodermal mid-gut, thus speeding up the formation of the complete gut in order to keep pace with the rest of development. In Cephalothrix the whole course of development is much slower than, for instance, in Drepanophorus or Prostoma, where even in the blastula stage the various 'Anlagen' are marked out. This point I consider to be of great importance in determining the affinities of the nemerteans, and will be discussed more fully at a later stage in this paper.

Proboscis.

Accounts of the direct development of the nemerteans agree that the proboscis rudiment appears very early—at least in the gastrula stage, while Lebedinsky (1897*a*) maintains that the 'Anlage' is recognizable in the blastula of Prostoma and Drepanophorus. This latter nemertean is the only form hitherto described, in which the development is not complicated by the proboscis entering into secondary connexions. In Stichostemma (Reisinger, 1926), Malacobdella (Hammarsten, 1918), Prostoma (Lebedinsky, 1897*a*) and Prosorhochmus (Salensky, 1884, 1909) the fore-gut and rhynchodaeum come to open into a common atrium.

In both the pilidium (Salensky, 1912) and in Desor's larva (Nusbaum and Oxner, 1913) the proboscis arises as the most anterior of the ectodermal invaginations appearing during the metamorphosis of the larva.

As a result of the characteristically slow and orderly development of the organ rudiments of Cephalothrix, the proboscis rudiment is not clearly indicated until comparatively late in development. It first appears in the newly-hatched larva (fig. 11 A, Pl. 21, *prb.*) as a few scattered cells, situated at the anterior tip of the body. The cells are roughly spindle-shaped, their cytoplasm is clearly defined, and they are obviously of a similar form to the ectodermal cells from which they originate. At first the separate elements arise more by immigration than by invagination, but later (fig. 17 A, Pl. 22, *rhynchod.*) an invagination which does not extend far into the interior, marks the anterior part of the rhynchodaeum.

In the oldest larvae which have been studied, the proboscis is represented by ectodermal elements extending posteriorly from the tip of the head between the dorsal and ventral commissures, at which point they migrate dorsally to lie over the top of the mid-gut (fig. 16, Pl. 22, *prb.*). The mesoderm of the proboscis is found merely as scattered cells which do not form a definite sheath round it; consequently there is, as yet, neither a proboscis muscular system nor a rhynchocoel.

Head-gland.

In the members of the genus Cephalothrix, the numerous elements of the head-gland open separately to the surface instead of through a common duct, as in many of the nemerteans. Bürger (1897) is of the opinion that the gland in Cephalothrix is not comparable to that of other forms, but Wijnhoff (1910) maintains that no such distinction can be drawn.

The 'Anlage' of the head-gland appears very early in directly developed nemerteans, and in some cases (Lebedinsky, 1897 *a*) appears to undergo a certain amount of degeneration later in development.

In Cephalothrix, the gland is restricted to the anterior region of the body in front of the brain (figs. 16, 21, Pl. 22, *hd.gl.*). After 3–4 days of free-swimming life, a few of the ectoderm cells in the head region of the larva lengthen considerably. They lose their cilia, and their contents, which are at first granular and of a brown colour (fig. 17 A, Pl. 22, *hd.gl.*), do not readily take up

stain. Later, however, the gland becomes filled with a substance staining deeply with iron haematoxylin, Delafield's haematoxylin and aniline blue. In this condition, each of the glandular elements is elongate and somewhat pear-shaped (figs. 16, 21, Pl. 22, *hd.gl.*), the tip projecting for a short distance beyond the limit of the ectoderm. The greatest concentration of glands is at the extreme anterior tip of the body, but a few open rather more laterally and posteriorly.

Cerebral Organs.

These organs are not present in the adult Cephalothrix, and are not represented at any time during development. The absence of cerebral organs is therefore not a secondary condition as in Malacobdella where (Hammarsten 1918) has shown that the two lateral invaginations, one on each side of, and a little posterior to, the blastopore, are the 'Anlagen' of the cerebral organs which later degenerate after having cut off a nervous portion which attaches itself to the ventral ganglia of the brain.

Nephridia.

Contrary to Bürger's (1897) statement that nephridia are lacking in the nemerteans of the genus Cephalothrix, Wijnhoff (1910) has demonstrated their presence in the three species Cephalothrix linearis, Cephalothrix filiformis, and Cephalothrix rufifrons. They open separately to the exterior through a duct which internally swells out into one or two vesicles ('Endkölbchen'), and which in Cephalothrix linearis, at least, has a ciliary flame.

No traces of nephridia are to be found in the larvae which have been sectioned, so that in this respect I have been no more successful than other observers of direct development. More evidence as to the origin of the nephridia in nemerteans is greatly needed, for in the pilidium, the nephridial rudiments have been described as outgrowths from the oesophagus, and on this account are considered by Bürger (1897) and others, to be of ectodermal origin. On the other hand, Nusbaum and Oxner (1913) derive the nephridia of the Desor larva of Lineus ruber from mesodermal elements.

Nervous System.

The nervous system of the pilidium is restricted to a marginal ring associated with the ciliated band, and it is not until metamorphosis that the rudiments of the adult nervous system appear. Dorsal and ventral ganglia arise from a single pair of 'Anlagen' of ectodermal origin, both in the pilidium (Salensky, 1914) and in Desor's larva (Nusbaum and Oxner, 1913).[1] In both of these developmental forms the 'Anlagen' are situated dorso-laterally in the anterior region. This condition is also found in some of the nemerteans such as Malacobdella (Hammarsten, 1918) and Stichostemma (Reisinger, 1926) with a direct development. The lateral nerve-cords arise, in all the above-mentioned forms, as posterior outgrowths of the ventral ganglia.

Lebedinsky's account (1897*a*), however, differs somewhat from the more general description in that he finds four separate 'Anlagen'—two furnishing the dorsal, and two the ventral ganglia. Moreover, these latter are only secondarily connected with the lateral cords, which arise separately from the ectoderm. The ventral, as well as the dorsal commissures are formed as a result of ectodermal proliferation; the ventral commissure develops definitively, but the dorsal connexion is suppressed and the definitive commissure arises secondarily by the union of the dorsal ganglia.

In Cephalothrix, nervous elements first appear in the gastrula as the two dorso-lateral invaginations previously described (fig. 7 A, Pl. 21, *ner.rud.*). At the close of gastrulation, however, it is impossible, for reasons given, to follow the rudiments further, and it is not until the larval stage is reached that the 'Anlagen' are at all clear. Fig. 11 B, Pl. 21, a parasagittal section through a young larva, shows the condition at this time. The dorsal ganglia (*d.g.*), situated just behind the 'eyes', are represented by aggregations of nerve nuclei; each nucleus is of a somewhat pear-shaped form with characteristically diffuse chromatin. Ventral, and slightly posterior to the dorsal ganglia,

[1] Bürger (1897), however, derives dorsal and ventral ganglia from separate rudiments, the former from the 'Kopfscheiben', the latter from the 'Rumpfscheiben'.

are the nuclei of the ventral ganglia (*v.g.*), and between the two, a few scattered nuclei mark the 'Anlagen' of the short lateral commissures (*l.c.*) connecting dorsal and ventral ganglia of each side.

The lateral nerve cords (*l.n.c.*) are formed as posterior outgrowths from the ventral ganglia and, as yet, are quite short. Around the base of the stomodaeum, numerous nerve nuclei (*stom.n.*) derived from the ectoderm of this region are the forerunners of the stomodaeal ('Schlund') nerve.

Although the origin of the dorsal ganglia is clear, that of the ventral ganglia is more obscure. It is certain that they are at least in part derived from the two original dorso-lateral rudiments, but it is at the same time probable that some of the elements of the ganglia originate from ectodermal cells of the stomodaeum region, but of that I must express a certain amount of doubt. The position may be summarized briefly thus: the nervous system in Cephalothrix arises primarily from two dorso-lateral rudiments first apparent in the gastrula. From these the dorsal ganglia and, as will be seen below, the dorsal and ventral commissures arise. The ventral ganglia are either simple or composite structures, in which latter case they are in part derived from nerve cells migrating from the ectoderm of the mouth region. The lateral cords appear as posterior extensions of the ventral ganglia. In general features, therefore, the nervous system of Cephalothrix resembles that of the directly developed Stichostemma and Malacobdella, as well as that of the metamorphosed Desor larva and pilidium, in its origin.

Even in the youngest larva the commissures are well developed (fig. 13, Pl. 22, *neurop.d.comm.*, *neurop.l.comm.*, *neurop.v.comm.*). The dorsal and ventral commissures are formed by union of their respective ganglia, and are distinguished chiefly by their neuropilem, which stains only very slightly with any of the reagents used, and is composed of a mass of fine interlacing fibrillae. The ventral commissure lies posterior to, and is better developed than the dorsal commissure. It is, however, the shorter of the two owing to the close proximity of the ventral ganglia.

Further development of the nervous system is confined mainly to the elongation of the lateral cords. Fig. 21, Pl. 22, a horizontal section through a 14-day old larva, at the level of the ventral ganglia, illustrates this. An aggregation of nerve nuclei around the stomodaeum presages the formation of the stomodaeal nerve (*stom.n.*), the connexion of which with the ventral ganglia is restricted to a few scattered nuclei. Similarly scattered elements (fig. 21, Pl. 22, *ceph.ner.*) in the head region represent the first indications of the cephalic nerves, but there is no trace of a dorsal, nor of a proboscis nerve.

Further History of the Larva.

Seven weeks after they have been liberated from the egg-membrane, the Cephalothrix larvae begin to show signs of degeneration. Not only do they become smaller, decreasing from a length of about 320μ to 250μ in the course of a few days, but the tissues become diffuse and difficult to interpret in section. Attempts have been made to find a suitable food for the larva, but neither Nitzschia[1] nor food material in various forms obtained from the sand in which Cephalothrix normally lives, were ingested. Indeed, I have not found food of any sort, apart from yolky matter, in the larval gut at any time during development.

Riches (1893) has observed the later development in larvae obtained from the plankton, and I can only add his account in order to complete the developmental history of Cephalothrix. He says 'Pelagic larvae were obtained from the tow net as late as December—they must therefore be at least 3 months old at this time. The largest of these was 3 mm. long, and was provided with an additional pair of marginal lappets, situated between the pair figured by McIntosh and Barrois and the anterior extremity. The eyes were situated at the margin of the head and relatively far back. Some days after the capture of this larva it gave up its pelagic life, and sank to the bottom of the vessel. About this time the eyes began to atrophy and very shortly the adult appearance was reached.'

[1] I have to thank Dr. E. J. Allen for supplying the culture of Nitzschia.

Discussion.

It has already been pointed out in the introduction to this paper, and has been noted by practically every recent investigator of nemertean development, that the pilidium and Desor's larva undergo a metamorphosis of a strikingly similar type. In both forms the adult ectoderm arises from two pairs of antero-lateral and postero-lateral amniotic invaginations and from a postero-dorsal unpaired rudiment derived by delamination (Salensky, 1914, Nusbaum and Oxner, 1913). Although most of the organ 'Anlagen' arise in similar ways in the two larvae, the origin and development of the stomodaeum and mid-gut present notable differences. It is true that in both cases the blastopore is carried into the enteron during gastrulation, but while in the Desor larva the invagination involves endoderm only, in the pilidium the invaginated tissue external to the blastopore is ectoderm. Consequently, the fore-gut of the pilidium is defined at an early stage, and communication with the mid-gut cavity is early established in that the blastopore remains permanently open. In the Desor larva, however, the blastopore closes and the originally endodermal fore-gut is replaced by ectoderm derived from a pair of thickenings on the hinder wall of the primary oesophagus just above the mouth.

The mid-gut of the Desor larva meanwhile undergoes a re-organization of the type found in Cephalothrix. By the absorption of reserve food material, the original syncytium is converted into the definitive single-layered gut, and communication between the fore- and mid-gut cavities takes place at the former point of closure of the blastopore.

Strictly speaking, the term larva (Desor larva) is used incorrectly in describing the development of Lineus ruber, for the whole of development is embryonic and takes place within the egg-membrane. In comparing the pilidium with Desor's larva, it is easy to understand how the detailed differences in the developmental processes have arisen. Lineus ruber lives under stones in the intertidal zone and is gradually becoming more and more adapted to conditions of desiccation, for at its upper limit of distribution it can remain uncovered

by sea-water for a period of from one to two days (Bürger, 1907). A habitat of this kind does not favour the production of a free-swimming pilidium, and consequently the embryonic period has been prolonged. Associated with this new set of circumstances, reserve material has been accumulated within the egg with resultant complication in the development of the mid-gut. It is, indeed, difficult to account for the production of a secondary stomodaeum on these facts alone, but in general features the differences between pilidium and Desor larva can be ascribed solely to environmental conditions.

Turning now to the question of the direct type of development in nemerteans. One finds, in all cases, an absence of a well-marked metamorphosis although the reserve food material originally present in the ovum necessitates a reorganization of the mid-gut cells at some stage in development. In certain respects, such as in the early development of the proboscis, head-gland, and cerebral organs, the direct development is sufficiently similar to that of the Desor larva or pilidium to call for no comment, while the origin of the nervous system from two dorso-lateral rudiments would appear to be of general occurrence. The only variant account among recent workers, in this respect, is that of Lebedinsky (1897*a*) who describes, in Prostoma and Drepanophorus, four separate rudiments. It is significant that in Malacobdella (Hammarsten, 1918), while the greater part of the nervous system is derived from the two above-mentioned 'Anlagen', nervous tissue associated with the cerebral organ rudiment (which later degenerates) contributes to the ventral ganglionic masses. In Cephalothrix (p. 368) it has been shown that there appears to be a similar migration of nervous tissue from the ectoderm of the stomodaeal region. Nevertheless, the two dorso-lateral rudiments must be regarded as the primary 'Anlagen'.

The embryology of Cephalothrix differs from all other nemerteans in which direct development has been described, in the slow and orderly way in which the various 'Anlagen' appear. Already in the blastula of Prostoma and Drepanophorus (Lebedinsky, 1897*a*) most of the rudiments are marked out, while in the 76-hour embryo of Malacobdella (Hammarsten,

1918) the proboscis, apical organ, nervous system and mid-gut rudiments are present. In the embryo of Cephalothrix at this stage only the gut and nervous system rudiments are represented. Moreover the hind-gut 'Anlage' in Malacobdella, Prostoma, and Drepanophorus appears during the gastrulation phase. In Prosorhochmus (Salensky, 1884, 1909) and Stichostemma (Reisinger, 1926), however, it is of late appearance, while in the 8-week old Cephalothrix there is not the slightest indication of a hind-gut, a condition reminiscent of the pilidium.

Diverse accounts are given of the derivation of the fore-gut in directly developed nemerteans. Salensky (1884, 1909) is alone in considering the fore-gut to be of endodermal origin. There, the mid-gut for a time exists as a closed sac lying within the blastocoel, and the fore-gut arises as a ventral diverticulum which eventually opens to the exterior at the point of closure of the blastopore.

In all other cases the stomodaeum has been described as ectodermal. In forms such as Malacobdella and Stichostemma, where in the adult both stomodaeum and rhynchodaeum open into a common atrium, the adult fore-gut is of secondary origin, the primary stomodaeum losing its connexion with the mid-gut and disappearing. It is the primary stomodaeum, however, which is the important structure from the comparative point of view.

The primary stomodaeum of Prostoma, Drepanophorus, Stichostemma, and Malacobdella arises in the neighbourhood of the closed blastopore, which in these forms is not pushed into the enteron, but remains for a time as the opening of the mid-gut cavity to the exterior. A stomodaeum arising some time after the closure of the blastopore, during which interval the mid-gut is a closed sac, must be regarded as a secondary condition when compared with the pilidium and Cephalothrix larva in which the stomodaeum functions from the time of its initiation as the definitive fore-gut.

We may thus divide the nemerteans with direct development into two distinct groups.

(*a*) The Cephalothrix Type.

i. There is no telescoping of the developmental processes, and the 'Anlagen' appear in a leisurely and orderly manner.

ii. The stomodaeum is formed at gastrulation.

iii. The hind-gut appears late in development and is probably of endodermal origin.

(*b*) The Enoplous Type[1] (Malacobdella, Prostoma, Stichostemma, Drepanophorus, &c.)

i. There is a telescoping of developmental processes in that practically all the organ 'Anlagen' are represented in the blastula or gastrula.

ii. The stomodaeum is formed as a separate invagination distinct from that involving the mid-gut, and may later disappear owing to the definitive fore-gut entering into secondary connexions with the proboscis.

iii. The hind-gut appears relatively early and is of ectodermal origin.

We have already (p. 372) had occasion to compare the direct development of Cephalothrix with that through the pilidium. Comparisons based on the details of the cleavage process and on the origin of the mesoderm are of little value, in the former case because of individual and specific variation, in the latter on account of our lack of detailed knowledge concerning the identity of the teloblasts. In the origin of the stomodaeum there is, however, as we have seen, a close resemblance between the two forms, if we admit that the oesophagus of the pilidium is of ectodermal nature—a view supported by the majority of the writers on the subject. Comparison of subsequent stages is made difficult on account of the metamorphosis of the pilidium; but, apart from the peculiar method of origin of the definitive ectoderm, associated with this process, the origin and development of the organ rudiments is sufficiently similar in the two forms not to invalidate comparison.

Further, the late appearance of the hind-gut is a feature

[1] Nemerteans with the more specialized type of direct development are enoplous forms, i.e. they have an armed proboscis.

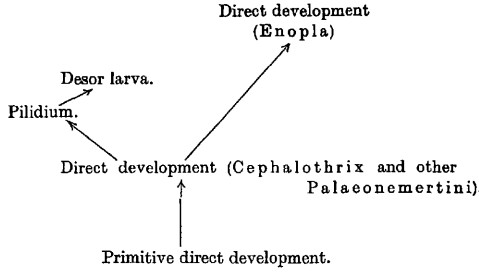
common to the pilidium, the Desor larva and the Cephalothrix larva, and is important in that it represents a primitive condition and is reminiscent of the gut of the Platyhelminthes, where the anus is at all times lacking.

With these considerations in mind, we cannot but conclude that the developmental forms of the Nemertea fall into two main groups. On the one hand there is the specialized type of direct development characteristic of the Enopla, and on the other, the more primitive direct development of Cephalothrix (and no doubt of other anoplous forms, particularly of the Palaeonemertini), the pilidium and larva of Desor.

In the latter group the pilidium and Desor's larva are closely related forms in which secondary developmental features have arisen in connexion with the early acquisition of the free-swimming habit. This being the case, Desor's larva must be regarded as being still more specialized, for it has re-acquired the embryonic habit in consequence of the habitat of Lineus ruber. Further specialization is reflected in the mode of formation of the stomodaeum, and in the relatively early appearance, as compared with the pilidium, of the hind-gut.

More remotely connected with the anoplous direct development is that of the enoplous forms such as Prostoma, Drepanophorus, Stichostemma, and Malacobdella. Although in one respect—the absence of a marked metamorphosis—these would appear to be more closely related to the Cephalothrix condition than is the pilidium or Desor larva, details of the developmental process show that in the method of formation of the stomodaeum and hind-gut and in the telescoping of development, this type of direct development is far removed from the primitive type. The scheme would be represented graphically as on p. 376.

This scheme, based on developmental affinity, agrees very well with that founded on adult characters. The Enopla are undoubtedly more specialized than the Anopla, and in this latter group, the Palaeonemertini, with the simple direct mode of development, are less specialized than the Heteronemertini in which the pilidium and Desor larva are the characteristic larval forms.



Summary.

1. Cephalothrix rufifrons is a nemertean with a direct type of development. The spherical larva which escapes from the egg-membrane, is equipped with the rudiments of most of the adult organs. The later larva is elongate and shows advance in the further differentiation of its tissues and organs and in the development of a head-gland.

2. Fertilization is internal, and the eggs, when laid, have a surrounding fertilization (egg-) membrane within which lie the two polar bodies.

3. Cleavage of the spiral type leads to the formation first of a spherical, and later of an elongate blastula in which macromeres and micromeres can still be distinguished. None of the organ 'Anlagen' are recognizable at this stage.

4. Gastrulation is effected by invagination, and involves both endoderm and ectoderm so that the blastopore is carried into the lumen of the gut to mark the channel between fore-gut and mid-gut. The blastopore subsequently closes and stomodaeum and mesenteron are separated.

5. The mesoderm arises as four groups of cells, presumably from four teloblasts situated antero- and postero-lateral to the blastopore and may probably be referred to the blastomeres $2a^{1111}$–$2d^{1111}$. Isolated mesenchymal elements of ectodermal origin are also to be found.

6. For a time the germ-layers are indistinguishable, but a

process of absorption of yolk and reserve food material precedes the differentiation of the three layers.

7. Owing to the confusion of the germ-layers at the time of appearance of the mesoderm, the true nature of the secondary body-cavity, whether it be a re-expansion of the blastocoel or a true coelom, remains a matter of doubt.

8. The embryonic stomodaeum persists in the larva after differentiation of the elements of its wall which are essentially similar to the cells of the general ectoderm. Long cilia project into the lumen, and unicellular glands with granular contents are formed from epithelial cells.

9. The mid-gut wall, after re-organization of its cells, becomes single layered and ciliated. Glands similar to those of the stomodaeum are later developed.

10. Communication between fore-gut and mid-gut is re-established at the base of the ventral pocket of the latter, at or near the point of closure of the blastopore.

11. A 'hind-gut' is not formed until very late in development, and it is considered to be endodermal and not in the nature of a proctodaeum.

12. The ciliated epithelial cells of the ectoderm are triangular in section, and from them are differentiated two types of gland cell, small unicellular glands distributed over the general surface, and large posteriorly placed mucus cells.

13. Two dorso-laterally situated invaginations arising during the gastrulation phase are the 'Anlagen' of the greater part of the nervous system. Dorsal and ventral ganglia and the commissures arise directly from these rudiments, while the lateral nerve-cords grow out posteriorly from the ventral ganglia. The stomodaeal nerve is at least in part derived from ectodermal elements of the stomodaeal region.

14. Immigration, and later, invagination of the ectoderm cells at the anterior end of the larva give rise to the proboscis rudiment and rhynchodaeum.

15. The pear-shaped head-gland elements are derived from ectodermal epithelial cells. At first their contents are granular, and do not take up stain, but later they are filled with a fluid deeply staining with the haematoxylins.

16. The formation of nephridia and cerebral organs has not been observed, and it is concluded, that the latter are at no time present.

17. The relationships of the various developmental types of the Nemertea are discussed, and the conclusion is reached that there are two types of direct development, one characteristic of the enoplous nemerteans and the other of the Palaeonemertini. The pilidium and larva of Desor of the Heteronemertini are more closely allied to the simpler direct development of the Palaeonemertini than to that of the Enopla. Of the four types the palaeonemertean development is the least specialized and is most reminiscent of the platyhelminth condition.

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EXPLANATION OF PLATES 21 AND 22.

Lettering.

bas.gran., basal granules of ciliated epithelial ectodermal cells; *bc.*, blastocoel; *b.c.*, body-cavity; *bp.*, blastopore; *b.p.'*, point of closure of the blastopore; *ceph.n.*, cephalic nerve elements; *ch.*, connecting channel between fore-gut and mid-gut; *d.comm.*, dorsal commissure; *deg.chr.*,

chromatin of degenerating nuclei; *deg.nuc.*, degenerating nucleus; *dig.*, tissue undergoing digestion in the lumen of the mid-gut; *d.g.*, dorsal ganglia; *d.l.h.*, dorso-lateral horn of the gut; *d.ridg.*, dorsal ridge of the gut; *e.*, 'eye'; *ect.*, ectoderm; *ect.gl.*, ectodermal gland; *ect.gl.,ect.gl'.*, ectodermal glands in different secretory phases; *end.*, endoderm; *epith.*, epithelial cell of the ectoderm; *flag.stom.*, stomodaeal flagellum; *f.m.*, fertilization egg-membrane; *g.v.*, germinal vesicle; *hd.gl.*, head-gland element; *i.o.z.*, inner, opaque zone of egg; *lat.flag.*, lateral flagellum; *lat.ridg.*, lateral ridge of mid-gut; *l.comm.*, lateral commissure; *l.n.c.*, lateral nerve-cord; *mac.*, macromere; *m.*, mouth; *mesench.*, mesenchyme; *mesod.*, mesoderm; *mesod. musc.*, mesodermal muscular elements; *m.g.*, mid-gut; *m.g.c.*, mid-gut cavity; *m.g.gl.*, mid-gut gland; *m.g.w.*, mid-gut wall; *mic.*, micromere; *musc.el.*, mesodermal muscular element; *musc.fib.*, muscle-fibres; *ncl.*, nucleolus; *ner.rud.*, 'anlagen' of the nervous system; *neurop.d.comm.*, neuropilem of the dorsal commissure; *neurop.l.comm.*, neuropilem of the lateral commissure; *neurop.l.n.c.*, neuropilem of the lateral nerve-cord; *neurop.v.comm.*, neuropilem of the ventral commissure; *nuc.*, nucleus; *nuc.*1–3, nuclei in successive stages of degeneration; *nuc.ect.*, nuclei of the ectoderm; *nuc.ect.mes.*, nuclei of ectodermal and mesodermal elements; *nuc.mes.*, nuclei of the mesoderm; *nuc.m.g.*, nuclei of the mid-gut cells; *o.p.z.*, outer, pellucid zone of egg; *p.b.*, polar body; *p.gran.*, peripheral granule; *post.muc.gl.*, posterior mucus gland; *post.mus.gl'.*, the same with mucus in the process of being exuded; *prb.*, proboscis rudiment; *prb.el.*, proboscis elements; *rhynchod.*, rhynchodaeum; *stom.*, stomodaeum (fore-gut); *stom.gl.*, stomodaeal gland; *stom.n.*, stomodaeal nerve; *stom.w.*, wall of stomodaeum; *v.comm.*, ventral commissure; *v.g.*, ventral ganglia; *v.l.h.*, ventro-lateral horn of the gut; *v.p.*, ventral pocket of the mid-gut; *v.ridg.*, ventral ridge of the gut; *y.a.*, region of yolk absorption.

Plate 21.

Fig. 1.—A. Unfertilized ovum taken from a mature female. B. Fertilized ovum with polar bodies and fertilization (egg-) membrane completely formed.

Fig. 2.—A. Section through a dividing egg in the plane of the first cleavage. B. Transverse section through an embryo in the four-cell stage.

Fig. 3.—Median sagittal section through a sixteen-cell blastula. Egg-membrane not shown.

Fig. 4.—Median sagittal section through a late blastula (27 hours). The egg-membrane is not shown.

Fig. 5.—A. Transverse section through the blastoporal region of a gastrula of 45 hours. B. Median sagittal section through a gastrula of 56 hours. The egg-membrane is omitted in both figures, and in A the cilia of the gut and general ectoderm are not shown.

Fig. 6.—Median sagittal section through a gastrula of 77 hours, at the time of closure of the blastopore. It is hardly possible to distinguish

between ectoderm, mesoderm, and endoderm. The egg-membrane and cilia of the ectoderm and gut are omitted.

Fig. 7.—A. Transverse section through a gastrula of 45 hours at the level of the nervous system rudiments. Cilia and egg-membrane omitted. B. Parasagittal section through a gastrula of 56 hours. Egg-membrane omitted.

Fig. 8.—Transverse section through a gastrula of 75 hours showing the process of absorption of yolk and degeneration of nuclear material. Egg-membrane and cilia of the ectoderm and mid-gut omitted.

Fig. 9.—Parasagittal section of a gastrula of 36 hours to show the disposition of two of the four primary mesoderm groups, antero- and postero-lateral to the blastopore. Cilia and egg-membrane omitted.

Fig. 10.—Section through the stomodaeum and mid-gut (slightly inclined from the horizontal plane) of an embryo immediately prior to escape from the egg-membrane.

Fig. 11.—A. Median sagittal section through a larva immediately after its escape from the egg-membrane (160 hours). B. Parasagittal section through a larva of the same age as A. The cilia of the gut are not shown.

Fig. 12.—A. Transverse section through the stomodaeal region of a larva of 160 hours. B. Transverse section of the same larva behind the stomodaeum. The cilia of the gut are not shown.

Plate 22.

Fig. 13. Transverse section through the dorsal and ventral ganglia of a larva of 160 hours.

Fig. 14.—Horizontal section through the extreme posterior end of a 4-weeks old larva.

Fig. 15.—Transverse sections through a larva 45 hours after rupture of the egg-membrane. Although the organ rudiments are well developed, the tissues are, as yet, owing to the abundance of yolk, not completely differentiated. A. Through the stomodaeum. B. Through the dorsal and ventral ganglia. The cilia of the mid-gut are not shown.

Fig. 16.—Median sagittal section through a 2-weeks old larva.

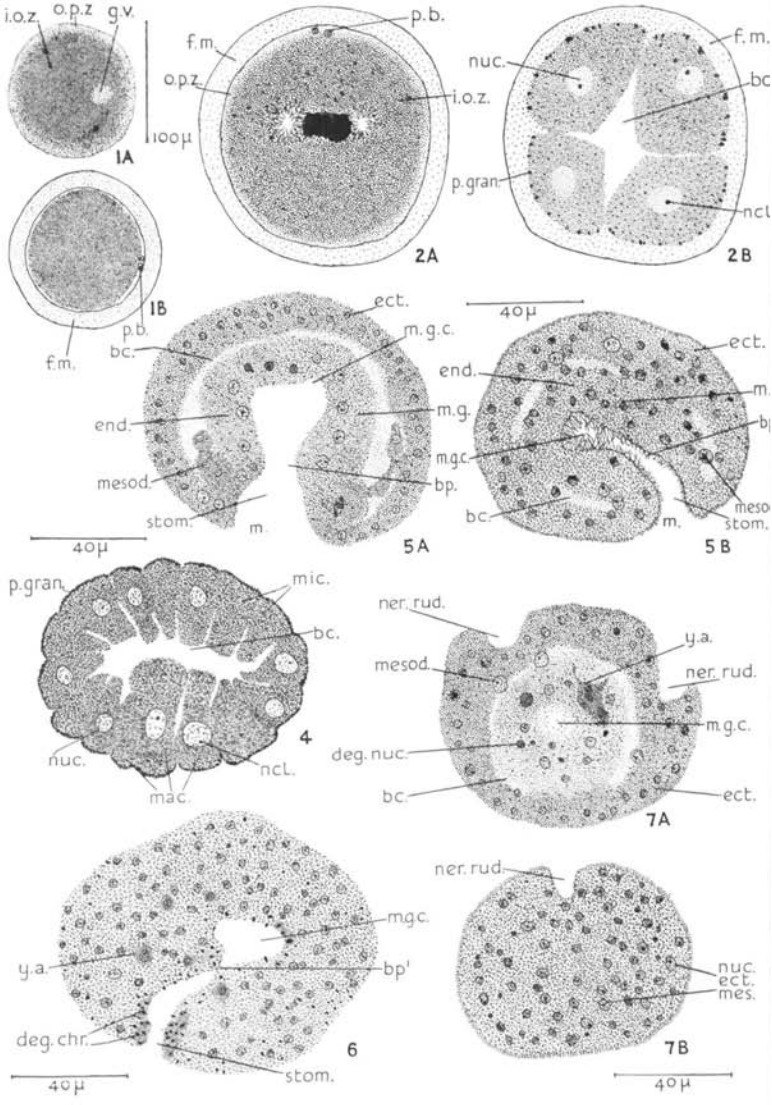
Fig. 17.—Transverse sections through a 26-day old larva. A. Through the extreme anterior end. B. Through the 'eyes' and ventral ganglia. The cilia of the mid-gut are omitted.

Fig. 18.—Transverse section through the stomodaeum of a 26-day old larva. The cilia of the mid-gut are not shown.

Fig. 19.—Transverse section just behind the stomodaeum of a 26-day old larva. The cilia of the mid-gut are omitted.

Fig. 20.—Transverse sections through a 2-weeks old larva. A. Through the middle portion of the mid-gut. B. Through the extreme posterior end. The cilia of the mid-gut are omitted.

Fig. 21.—Horizontal section through a 2-weeks old larva at the level of the ventral ganglia. The cilia of the mid-gut are omitted.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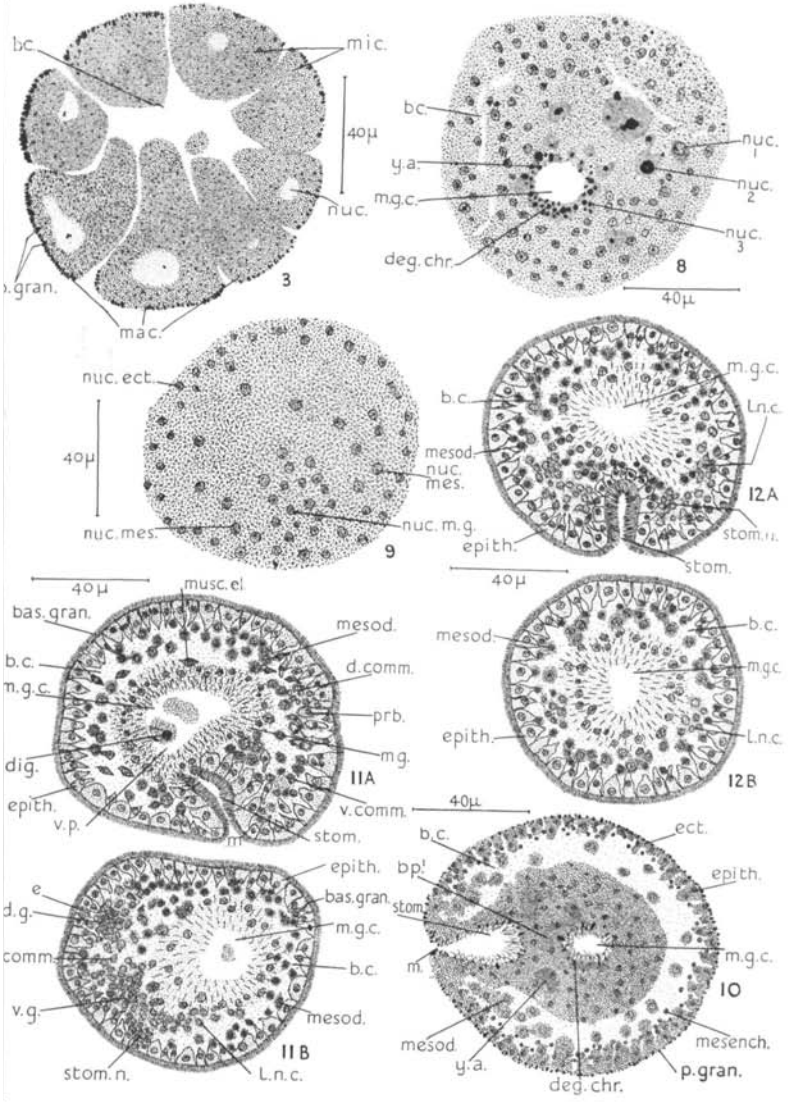
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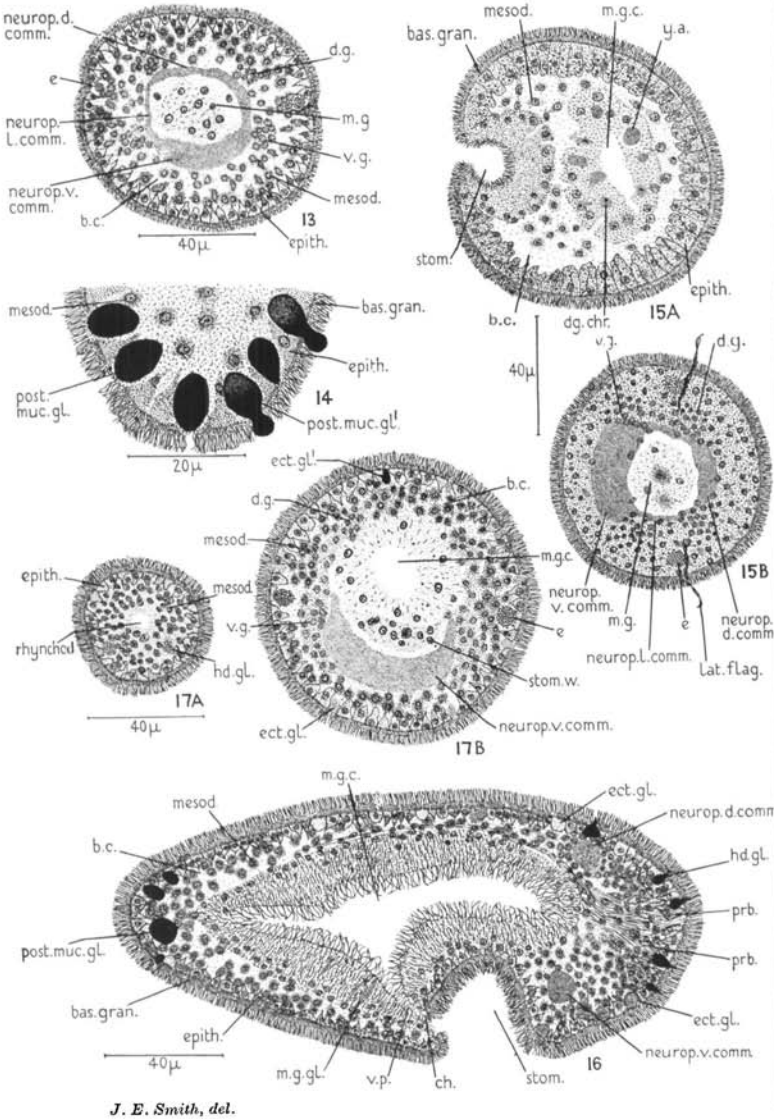
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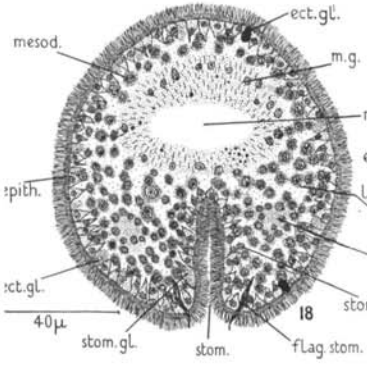
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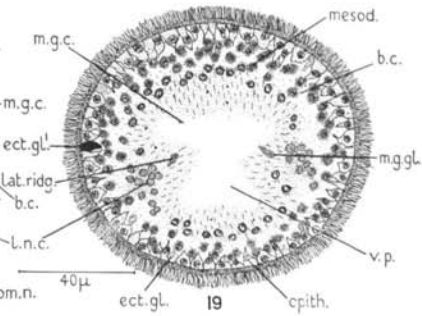
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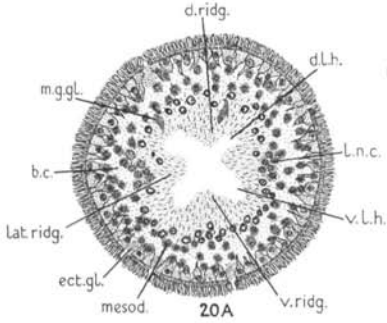
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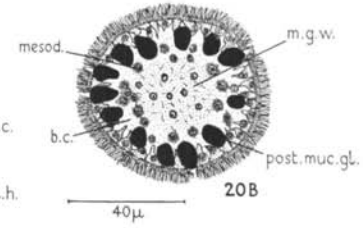
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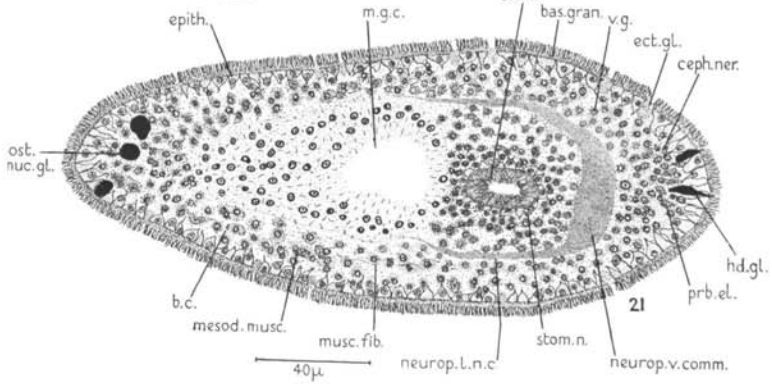
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