THE ANATOMY, AND THE HISTOLOGY OF BUD-FORMATION IN THE SERPULID *Filograna implexa*, WITH SOME CYTOLOGICAL OBSERVATIONS ON THE NUCLEI OF THE NEOBLASTS.

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CONTENTS.

The present piece of work has arisen from a suggestion made to me by Dr. E.J.Allen, Director of the Marine Biological Laboratory, Plymouth, that I should investigate the histology of bud-formation in Filograna. As the work progressed, it was felt to be very unsatisfactory to be continually handling an animal, and to be concentrating so much attention on minute internal details, while its general structure and behaviour were so little known. There is a certain amount of previous literature on the Serpulids which helped considerably, but there is no complete account of any one genus.

MEYER'S paper (1888) is the earliest work on the anatomy of the family, and since then there has been the paper by LEE (1912); neither of these pays particular attention to Filograna. The authors who have dealt at greatest length with this particular genus are McINTOSH, and MALAQUIN. McINTOSH gives the most complete recent account of the genus in his monograph of 1923, including not only his own previous work, but also a full historical survey and bibliography: and account of the distribution of the genus is also given, and details of the form of the colony and of the chaetae. MALAQUIN'S work is spread through a large number of small papers, which are referred to individually in the text subsequently.

The combined results of these two authors still do not make a complete account of the anatomy of the animal. MALAQUIN'S results approximate in some points very closely to the present author's observations, particularly in the histological section of this paper, but it is considered that there is sufficient difference to justify entirely an additional publication on the genus.

The present paper is an attempt to give an account, which, though it cannot approach completion, will be sufficient to be a useful basis for anyone working on this, or on allied species, in future. The information already available on the structure, the habit of life, and the life history, has been collected and added to: particular attention has been paid to all that has a bearing upon bud-formation.

In the previous papers, especially those of MALAQUIN, there is an almost complete lack of illustrations - therefore particular care has been taken to make the figures adequate.

Filograna proved to be particularly suitable as the subject ~~to~~ for a monograph of this nature for several reasons: it is a British form (cosmopolitan in fact,) both abundant and easily obtainable; also the small size of the animal makes it possible for one investigator to carry the work to a stage of greater detail than would be possible if the animal were either larger, or more complex. The interest of the subject is greatly increased by the fact that *Filograna* has evolved a method of a sexual reproduction, which in its biological significance, seems to be unique.

Bud-formation under conditions of sedentary, tubicolous existence, is very satisfactory for at least two reasons: the whole process occurs within the shelter of the tube, and involves no dangerous free-living phase: also it results in a colonial habit - which is favourable to sedentary forms. It is probable that the habit of asexual reproduction is responsible for the great success and ubiquitous distribution of the species.

A brief survey of budding in Annelids shows it to be a phenomenon characteristic of the Syllids and of two families of Oligochaeta, namely, the Aeolosomatidae~~:~~ & the Naididae: otherwise it is confined to isolated genera; budding in one individual *Eulalia* is recorded by VERRILL (1873) and in *Myxicola* and *Potamilla* among Cryptocephala, by CAULLERY and MESNIL (1920).

The buds in Syllids, in Oligochaetes, and in *Filograna*, are not all similar, or even equivalent to each other, though described by the same general term "buds." The budding in Syllids has been the subject of a considerable amount of work, particularly by MALAQUIN (1893) and ALLEN (1921 and 1927). The buds in this case are essentially sexual and are concerned with the dissemination of sexual products: the division between bud and stock coincides more or less accurately with the division between genital and non-genital regions of the body, and the actual separation of the sexual zone as a bud, is really to be regarded as a case of epigamy carried to the extreme.

In oligochaetes the budding is of a different nature: it may here be accurately described as asexual reproduction, inasmuch as it is a means of increasing the number of individuals of the species, by fission of one body into two, more or less similar to each other. It is in many genera much more in evidence than the sexual method of reproduction, and it appears that it is much economical in output of energy than the sexual method.

The biological significance of the buds in Filograna is rather greater than it appears to be in the Oligochaeta (see p 24). It is again a true~~r~~ multiplication of individuals, and the two individuals formed by fission of the stock, are again essentially similar.

The relationship between sexual and asexual phases is in this case somewhat inconstant. The budding individual generally shows no sexual organs, but in cases in which these are visible, they are confi~~m~~ned to the stock, or parent individual, and are generally less abundant than in a mature non-budding individual. The sexual and asexual phases appear to be quite independent of each other, and they may overlap, or may follow each other in sequence. The conditions in fact, are more similar to those seen in Oligochaetes than to those in other Polychaetes, and the possible variations in the case of Filograna may even be compared with parallel cases in different genera of Oligochaetes: in Ophidonais, the sexual and asexual processes are mutually exclusive, while in such general as Naïs and Chaetogaster, the two overlap more or less. A similar comparison with the Oligochaetes is seen when the variability of the position of origin of the bud on the parent individual is considered. (see p 25).

The fact that buds occur in Filograna has been known for nearly a century, and the buds have often been mentioned, and even figured since then. This is to be expected as the species is so abundant, and as the buds are produced more or less ~~prdeficdally~~ prolifically through about eight months of the year.

SARS (1846) described them for the first time, and gave considerable detail of their external form. His original words were as follows: "Im Monat April 1839 untersuchte ich wieder diesen Ringelwurm, und machte dann die höchste interressante Entdeckung dass er sich durch Proliferation fortpflanzt. Schon während meiner oben erzählten Beobachtungen fielen mir bei einem Individuum einige sehr kurze und dicke Fäden auf der Mitte des Hinterkörpers auf - - - - Ich erkannte zu meiner grossen Verwunderung dass sie von einem aus dem Hinterkörper unseres

Ringelwurmes hervorwachsenden Jungen her-ruhren."

Since this date the buds have genefally been mentioned in descriptions of the genus, and it is of interest to note that HUXLEY mentions, and figures, them in his text book - The Anatomy of Invertebrate Animals - 1877, under the name *Protula Dysteri*.

There is one controversial point in connection with *Filograna* which has been raised by most of the previous authors and which must be raised again, namely, the classification of the two species *Filograna implexa*, Berkeley, and *Salmacina dysteri* (Claparède). McINTOSH concludes, and the present author agrees, that there is insufficient reason to justify the separation of these into two genera. The chief difference between them lies in the fact that *Filograna* has its two dorsalmost branchiae modified to form opercula, and *Salmacina* has not. McINTOSH bases his conclusion on the variability and inconstancy of the diagnostic opercula, and below is given evidence of the variability of other features, both external and internal. However, McINTOSH'S decision is apparently not universally accepted. The two earliest authors on the genus raised this question first. SARS describes the presence of opercula - and SCHMIDT two years later (1848), described a type without opercula: however, he mentions that he has seen other samples possessing opercula, and concludes that "das Merkmal ist also, in meiner Species wenigstens, nicht constant, und wird eliminirt werden müssen." That remains the essence of all that can be said on the subject even now. In the two most recent publications on the Serpulids, one (FAUVEL, Faune de France. Polychètes sédentaires. 1928) retains the two genera, and the other (BENHAM: British Antarctic "Terra Nova" Exp: 1910, Vol. VII No. 2, 1927), unites them under the name *Filograna*. One of the diagnostic features given by FAUVEL is quite contrary to all observations recorded in the present paper: he states that in *Filograna* the sexes are separate, while *Salmacina* is hermaphrodite. Apart from this erroneous statement, the distinction is based on the slight differences in the form of the branchial filaments. MALAQUIN uses both specific names but does not discuss the question.

There is equal uncertainty in regard to the classification of the other species of *Salmacina*, viz; *S. aedificatrix* and *S. incrustans*: BENHAM includes them both in *F. implexa*: FAUVEL includes *S. aedificatrix* but retains *S. incrustans*. The present author is of the opinion that *S. aedificatrix* should be suppressed, but has been unable to see *S. incrustans*. The samples obtained from Naples, where LO BIANCO (1893) records *S. aedificatrix*, differed in no way from some of the varieties obtained in England. Whether this really was his *aedificatrix* material or not, is not quite certain as the means of distinguishing the two are so slight. It was found that LO BIANO'S record of these forms is not accurate, as he does not mention *Filograna*, and records both the Neapolitan forms as *Salmacina*, defining this as having no opercula. However during a recent short stay at Naples two samples of material were obtained for me by the courtesy of the Director of the Station, and one of these was of the operculate variety, and one non-operculate. To repeat what has already been stated, it is my experience after having examined samples from such scattered collecting grounds as Plymouth, Aberdeen, Naples, Roscoff and Norway (these last two collected for me by Mr.N.J.Berril), that there is only one species.

The operculate and the non-operculate varieties both occur in the Plymouth area, and both were examined in large numbers. Material obtained from the town pier is of the typical non-operculate type, and that obtained from deeper water on the Mewstone ground, operculate. But other samples taken inside the Sound, on Winter Shoal and the G.W.R. Docks, contains both varieties.

There are also other differences apart from opercula found, when samples from several collecting grounds are compared. For instance the "Salmacina" colonies on the pier are bright red in colour, as are those found in deep water at Revelstoke Point, while those on the other grounds mentioned (producing pure "Filograna" or the mixed colonies) are brownish, as a result of the absence of the red pigment. The presence or absence of pigment has no relation to the depth at which the animals are growing: these collections have all been made between low water and a depth of about 15 fathoms, a comparatively small range.

A second feature which is variable, is the size of the thorax. The number of thoracic segments varies among the individuals of one colony within a range of about four segments (round a normal of seven).

Thirdly, the form of the colony varies: in one case there are encrusting groups of tubes, and in another, large fenestrated masses 10 or 15 cm, in diameter. Between these two extremes are gradations, not due to the age of the colony, but characteristic of the area, and of the substratum to which the tubes are attached. The non-operculate "Salmacina" forms massive clumps attached to rocks, the operculate form on the Mewstone is always found encrusting on *Lepralia*, and the mixed colonies are usually intermediate in size and attached to - or embedded in - sponges and other sedentary animals.

One fact which may help to account for the small size of the Mewstone colonies, is that a certain

Monstrillid parasite, Haemocera, is abundant here. Many of these tubes when broken are found to be empty, so it may be that the parasite diminishes the vigour of the colonies and prevents them from attaining a greater size. The reason why the parasite is restricted to one locality is not known. It may be a geographical one, but even if it is physiological, and taken as evidence of specific difference, it still does not seem to justify the two genera. The life history of Haemocera has been described by MALAQUIN (1901), and his account has been found to agree in every point with the observations made at present. The whole series of internal stages has been seen, but these have not been figured as MALAQUIN'S paper is fully illustrated.

Further, to return to the account of variation in the species, there are several ways in which the branchiae themselves may be modified, and the presence of an operculum on the end of a filament does not seem to be any more significant than the presence of any other particular kind of termination. The number of pinnules may vary between 12 and 24, and these may be all more or less equal, or may diminish in size distally. Also, some filaments possess a rounded swelling at the base of each pinnule, while others, on the other hand, have similar glandular cells forming a row up the outer face of the whole axis. The termination of the filament, (see Text fig. 1.), is sometimes a small rounded tip, smaller than the smallest and most distal pinnule, and sometimes is enlarged and opaque, swollen with glandular cells. The enlargement of the tip may be confined to the two dorsal filaments, or it may apply to all the eight equally. Two extremes of these conditions of size are as strikingly different as an operculate and a non-operculate filament ever are. It is not suggested that the opercula have any function other than that of closing more effectively the mouth of the tube, and if such a character be made of generic importance, it is of an entirely different order of magnitude from that of the differences which separate the other genera of ~~Super~~Serpulids.

The presence or absence of opercula does not seem to show any correlation with the variation of other organs; these data are therefore interpreted as supporting the conclusion reached above, that the two forms <u>Filograna implexa</u> and <u>Salmacina dysteri</u> are identical.

EXTERNAL FEATURES.

i GENERAL.

When the worm is in its tube and expanded, a circlet of colourless branchiae, the bright red head and two or three anterior somites are protruded from the opening, but on the slightest stimulation, the whole is rapidly withdrawn.

The body measures between 1mm and 4mm in length and has an average diameter of ·3mm. At the anterior end of the body is a branchial funnel formed of 8 pinnate filaments, commonly 0·8mm to 1·0mm in length, but in a particularly handsome variety obtained from Revelstoke Point, they attain a length of 1·8mm, i.e. the same length as the rest of the animal: in a sample taken at Naples, they even exceeded the length of the rest of the animal considerably. The body itself is divisible into three zones. Anteriorly is the thorax, which contains generally eight or nine segments, though any number between five and eleven occurs in the adult. The length of the abdomen depends upon age and maturity: it may contain as few as six, or as many as forty segments. There is an intermediate zone, consisting of two segments which have lost their parapodia, and this is referred to as the achaetous zone. Posteriorly the body terminates in two lobes, the anal papillae.

There are structural details in which the thorax and abdomen differ, concerning colour, presence of a membranous outgrowth, form of parapodia and distribution of cilia.

The red colour which tinges the colony, is almost entirely confined to the thorax, particularly to that part which is protruded from the tube where the animal is expanded. Further, the dark brown colour of the anterior gut is seen through the walls of the thorax. The abdomen is comparatively pale and clear, and owes what colour it has, to the presence of granules in the gut, to blood in the sinuses, and to the genital organs.

The thorax bears a membraneous outgrowth, the thoracic membrane, which has the form of two longitudinal folds, one on each side dorsal to the parapodia. They are united posteriorly by a small transverse ventral fold, and anteriorly by another such fold, immediately behind the branchiae. This latter, the collar, is enlarged, particularly at the angles where the lateral and ventral parts are confluent. The collar is inserted pointing forwards, and has this position when the animals is in its tube, but is reflected over the aperture of the tube when expanded. The thoracic membrane points obliquely outwards and upwards when out of the tube; when withdrawn, the two halves overlap and form a tubular, supra-thoracic canal. (See Text fig. 2). This membrane arises in the position of, and is probably homolgous with, the dorsal cirrus of other Polychaetes. The collar is merely the enlarged anterior end of the membrane, the two halves of which have grown round the sides of the first segment and fused ventrally.

The membrane is supported by the long chaetae of the thorax, which arise in the dorsal lobe of the parapodi~~um~~a:

in the ventral lobe is a row of hooked uncini. In the abdomen the arrangement is reversed, the long chaetae being ventral and the uncini dorsal. The lobe bearing the long chaetae in the case of the thorax, is long and slender, merely an investment of the base of the bristles: the ventral lobe has the form of a thick transverse ridge in which the uncinci are embedded. In the abdomen the neuropodium is smaller, and the notopodium is almost vanished. In the anterior abdominal segments there is another lateral finger-shaped outgrowth immediately behind th uncinal lobe of the parapodium: this forms a tubular canal, the coelomoduct, (c.f. Pl. 4 fig. 8), and when the segments are filled with the maturing sexual cells, these tubes enlarge, acquire an opening to the exterior, and act as the genital ducts: they become ciliated at this time. Behind the genital somites these tubes diminish in size, and at the extreme hind end are entirely absent.

The long chaetae are of two patterns: one has tapering capillary shafts, and the other has flattened toothed blades near the tip (described and figured by McINTOSH 1923). The first pair of bundles contain~~s~~ both kinds, a group of 4 to 6 capillaries, and another group containing about the same number of the complex type. The average length of these is ·3mm. There are no uncini on this somite, but its long chaetae are the longest in the body: they support the enlarged angles of the collar. The following somite has both long chaetae and uncini, but in this case the long ones are all capillaries and in one bundle, numbering about six. This segment bears a row of 20 to 30 uncini, inserted with their teeth pointing forwards, and the small hooks backwards. All the following thoracic somites are provided with both kinds of long chaetae, and uncini. They have a group of 3 to 5 of the simple form, and another of 2 or 3 complex, all measuring about ·2mm. In the abdominal segments, the long chaetae are reduced to 1 or 2 per parapodium, and have a length of ·12 mm only. Similarly the uncini are reduced in number also, 5 per segment being the average in the abdomen.

The intermediate achaetous zone is derived from the two anterior abdominal segments by the loss of their appendages. Firstly the chaetae disappear, then the parapodial lobes and coelomoducts.

There is a difference in the arrangement of cilia on the anterior and posterior segments. On the abdomen the ventral surface is ciliated: the ciliated cells are scattered evenly along it, and are accurately confined to that area of the epidermis which lies beneath and between the two ventral nerve cords: in addition there are tufts on the anterior faces of the notopodia, close to the openings of the coelomoducts. On the thorax, cilia occur on the dorsal surface only, on the body wall between the insertion of the two halves of the membrane, and on the dorsal surface of the membrane itself. In the achaetous zone, the intermediate lateral walls are ciliated, viz, the area between the nerve cords and the dorso-lateral lines: hence there is a ciliated band parallel to, and just posterior to, the hind margin of the thoracic membrance. The significance of this in connection with the course of the ciliary currents is demonstrated later. (p. 19).

Anterior to the segmented part of the body is the prostomium, projecting in front of the collar as a rounded lobe, and terminating in a small median papilla (Pl. 5 fig12). This papilla is ciliated dorsally, and bears at its tip the opening of the duct of the glandular sac. The head carries the eyes, two oblique rows of dark masses which sometimes appear structureless, and sometimes as distinctly cup-shaped. In the larva, when there is a single pair only, the fact that the pigment is really ranged round a hollow cup is most clearly seen: in the adult there is on each side a row of about six. (Text figure 12).

Ventral to the head is the proboscis, a long non-retractile tube, terminating in a slightly funnel-shaped opening - the mouth - and extending a little anterior to the tip of the head. It is separated from the head by a deep groove, (Pl. 4 fig. 9), which is closed laterally by the semi-circular base of the branchiae. The common branchial base is united with the sides of the head dorsally, and with the proboscis ventrally, and in both cases the line of junction is perfectly distinct and sharply defined, although it is not a habit of this animal to break off its branchaie autotomously. The junctions dorsally are two oblique lines as in Pl. 1 fig. 1, and ventrally are two lines parallel to these, reaching towards the middle line as far as the longitudinal proboscis muscles. Posteriorly they reach almost as far as the insertion of the collar. The clearness of the division is due to the fact that the head is strongly pigmented, while the branchial base is formed of clear transparent cells.

Division into four filaments on each side, occurs at the level of the tip of the proboscis, and each filament is provided with two rows of lateral branches, the pinnules, arranged alternately. Arising at the angles of the head, between the bases of the dorsalmost filaments, is a pair of ciliated finger shaped tentacles, overhanging the mouth opening. Between the bases of the pinnules, along the sides of the filaments, are patches of opaque cells swollen with globules (Text fig. 1): in some cases these are scattered along the external face of the filament. When stained with Mayer's muci-carmine, these cells show a pale pink colour.

The termination of the main axis, i.e. the region distal to the last pinnule, sometimes is merely a small rounded tip, and sometimes is thickened and greatly elongated (Text fig.1): one example measured .5mm on a filament of total length 2mm. These swollen tips are packed with a mass of opaque cells similar to those just mentioned, In other cases the two dorsal filaments have their tips modified to form opercula. In the development of these, the end of the axis becomes flattened, then the edges are rolled ~~back~~ dorsally and fused together, so that a cup-shaped structure results.

The whole inner face of the axis and pinnules is ciliated (Pl. 3 fig 5.) and the current produced by the cilia is the food capturing mechanism. Each pinnule~~s~~ bears a band of short cilia, which is fringed with long laterals - hence a virtual groove results. This leads into the collecting axial groove, which has a zig-zag course down the filament. The four grooves of one side unite at their bases, and lead directly into the sides of the proboscis, likewise ciliated.

In the interior of these filaments is a tubular cavity in which a blood vessel lies: these are described in the section dealing with "Internal Structure", but they can be seen in the living animal perfectly plainly.

When the animal is expanded, the branchiae are held in such a position that the two dorsalmost lie in the same straight line as the tube itself (Text fig. 2): hence the funnel is not arranged symmetrically round the tube opening, but ventrally to it. The cilia produce a centripetal current which is directed accurately into the mouth. It does not cause any water to enter the tube, in fact there are tufts of cilia on the reflected face of the collar which specially guard against this.

The course of currents inside the tube is determined by the cilia on the body wall and on the head. The tip of the head is ciliated dorsally, and there are two rows of long cilia at the back of the head (Pl. 1 fig. 1), one being actually on the head (cil. b.1.) and one on the first thoracic segment between the insertion of the two angles of the collar (cil. b.2.). The current of the tube enters posteriorly, passes over the surface of the abdomen, then the more ventral current is collected dorsally round the hind edge of the membrane, and all passes out of the tube via the supra-thoracic canal. The course is seen by taking a small piece of colony, or a few individuals in glass tubes, and adding finely ground charcoal or stained starch grains to the water.

Probably the thoracic membrane is of use in helping to direct this current, as it effectively confines the outgoing stream in the thoracic region, to the supra-thoracic canal: in fact any movement of water on the ventral side of the thorax is in a posterior direction, and is due to the suction action of the main current flowing round the hind edge of the membrane. The necessity for protecting this region from a strong outward flow, is probably accounted for by the presence of a large mucous gland underneath the collar and first thoracic segments - the gland shield (see p66 whose secretion would at once be washed away.

The tube current will aid respiration, and also it disperses the anal excretion. The anus is terminal but directed dorsally, so that the faeces are carried forwards to the anterior opening of the tube. Here they are scattered, chiefly as a result of the beating of the head cilia. In some cases the tube is notched dorsally, and when this is so, the faeces are even more effectively removed. The spermatozoa on being discharged from the body, are likewise carried out of the tube immediately by this current: the forward flow is seen when the ripe and discharging animals are watched even after having been removed from their tubes.

The maintenance of the ciliary currents is a matter of vital importance to the animal, and is intimately correlated with its external form: hence the direction of the flow is necessarily similar in closely related species. SOULIER in 1891 described the course of the currents in the tubes of other Serpulids, and found it to be exactly as has been described in <u>Filograna</u>. When speaking of the thoracic membrane, he says: "La présence de ces organes" (les bouchiers et la collerette) "est elle en relation avec le séjour de l'Annélide dans le tube?" This question has been answered in the previous paragraphs, in the affirmative. It is interesting to note the fact that the current in the tubes of Sabellids is also in an anterior direction: this is shown to be so by the fact that when <u>Sabella</u> are kept in an aquarium, the faecal matter is discharged from the anterior opening of the tube and collects on the floor of the tank immediately beneath it. What difference the absence of thoracic membrane makes, has not been ascertained. A comparison between Sabellids and Serpulids in this respect would no doubt throw some light on the exact nature of the relationship that was suggested by SOULIER to exist between the presence of the membrane and the tubicolous habit.

Very similar in appareance to large stiff cilia, are the palpocils. These are distributed singly over the dorsal edge of the collar, and on the membrane and the tips of the branchial pinnules.

EXTERNAL FEATURES . 2. Phases of life history.

The description of external features must be supplemented by mention of those features which distinguish the various stages of life history. The foregoing description may be taken to apply to a young individual, after the time when it first acquires the typical adult features and before it shows the first signs of reproduction. In addition to such individuals, some are found bearing a bud at their hind end, and others show signs of preparing to produce a bud, or of recovering after having done so. The presence of sexual organs also modifies the appearance: they may be male only. or male and female both (in varying proportions): this applies to the budding and non-budding worms equally. Also, eggs and embryos up to the age of settling are found in the tubes of the parent in great abundance during the summer. The statement of DE ST. JOSEPH (1894) that they are found inside the body of the parent - that it is in fact viviparous - seems to have no foundation. Hence every stage of the life history is obtained by breaking up a [redacted] small piece of the colony.

The relative proportions of these different forms depends somewhat on the time of year, the buds being more abundant during spring and early summer (February to July at least), and sexual forms during the summer and autumn. Budding can continue all the year, for even in mid-winter (December) a few isolated buds have been seen. During March, April and May buds are extremely abundant: in the following three or four months buds are less abundant and the sexual forms mature. (At Naples a sample taken on April 20 1928) contained individuals with sexual organs practically mature). After the sexual phase there follows a period of reduced activity during which buds are rare. In the summer when budding and sexual reproduction are both possibilities, it is generally found that members of one colony tend to synchronise, and pass through the same phase at the same time, though this is never strictly the case. A consideration of other Polychaetes suggested that the sexual organs might have a monthly rhythm coinciding with that of the moon, and in order to test this, samples were collected regularly during a period of one month. The results obtained were negative, as the proportions of sexual and asexual individuals did not vary in any marked manner.

The duration of the life of the stock, and the number of asexual generations which may intervene between any two sexual cycles, are two points which have not been determined, since observations are so difficult to make on tubicolous animals. Probably each individual has a sexual cycle at least once a year, but how many years an individual stock can last, is unknown. There is evidence that an animal lasts into at least a second spring and begins budding again then (See page 42). This arrangement, by which the sexual and asexual phases alternate, is different from the condition seen in the Syllids - where the stock is asexual and the buds sexual, and doomed to perish, and also from that seen in the Oligochaetes - where numerous generation of buds are produced and sexual phases are rare. To anticipate the description given later in the histological section, it may be mentioned here that when a bud is produced, the tissues of the somites concerned are completely disintegrated and replaced by fresh ones. This process of histolysis is not confined to the bud, but extends throughout the tissues of the stock (Pl. 2 fig. 2K.) Hence there seems to be a rejuvenation of tissues in the stock as well as in the bud at this time. In the Oligochaetes no comparable process has ever been described, but in view of the fact that they are capable of producing very large numbers of asexual generations in succession, without the interpolation of any sexual process, it would be of great interest to know whether any such mechanism for rejuvenation exists.

EXTERNAL FEATURES. 3. Configuration of buds.

The buds produced by <u>Filograna</u> are terminal and single, and remain attached to the parent stock until all external developments and changes are complete, and the bud has the appearance of a normal adult.

They are very variable in size when young, the number of initial segments varying between extremes of 3 segments and about 20 segments: when older there is much less variation, as proliferation of segments posteriorly compensates for any original lack. The position on the abdomen of the stock at which the bud is separated is equally inconstant: the various possibilities are discussed in the following section of the paper.

The processes involved in the formation of a bud, have been partially described by MALAQUIN (1895 and 1905) and as he states, they include both a transformation of old somites, and a growth of new tissue. The head, branchise, collar and next thoracic segment, are all formed from new tissue proliferated anteriorly at the zone of separation of bud and stock. The following somites are formed by adaptation of the original terminal segments of the stock, while the most posterior segments are produced by the growing point at the posterior end in the usual manner, after the commencement of the formation of the bud. Hence there are two zones of active growth in a budding animal, one terminal, and one at the level of the origin of the bud head. The

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* The nature of the plane of [illegible] [illegible] considered on page [illegible].

latter not only produces the anterior part of the bud, but also a varying number of new somites anterior to itself: these will be the posterior segments of the stock after separation of the bud. The possibility of active growth taking place at a point removed from the hind end, is a common feature in budding and in regenerating Annelids. Strictly speaking, two separate processes of proliferation are in action at the plane of fission, as is show below (page 148), one on the stock and one on the bud.

The first visible sign of the production of a bud (pl. 2 fig.2.) is the appearance of a pair of opaque masses lying at the sides of the gut, and extending throughout all the segments which will be concerned. These are formed by aggregations of both phagocytic and embryonic cells. Sometimes they extend even further forwards gradually diminishing in size, into the somites which will remain in the stock. The transparency of the normal abdomen makes these conspicuous to the naked eye. Later a pair of prominences appear dorsally, the rudiments of the branchiae, and the appearance of these determines the line of division between bud and stock.* The lobes increase in size, and each becomes bi-lobed: the right and left pairs then migrate laterally remaining connected together by a transverse ridge of tissue - the rudiment of the new prostomium. Still later a third lobe appears on each side, and these three filaments

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* The relation of the plane of fission to septa, is considered on page 125.

elongate considerably before the fourth, ventral-most arises. At first the fourth is a rounded swelling at the base of the third, but later it becomes equal in size to the rest. From their first appearance, these branchiae are greenish, the colour being due to the presence in them of a broad blood sinus.

Other less obvious, but equally important changes take place in this region, concerning the ventral and lateral portions of the new head. A circular constriction separates the bud from the stock, and posterior to this, the new tissue laid down forms a complete ring which, as it increases in size, separates further and further ~~forms a complete ring, which further~~ those two planes, now one on the stock and one on the bud, which at the beginning of the transformation were adjacent. The most anterior of this new tissue forms a ring projecting forwards and encircling the hind end of the stock dorsally and laterally, but remaining very small ventrally, where, as mentioned below, the anal papillae of the stock develop, projecting backwards. See in lateral view (Pl. 2 fig. 2E), there is an overlap of the two individuals here, and a corresponding deep grove between them. This separation involves only the external tissues, as the gut and longitudinal muscles are continuous (though constricted) till the very end. The separation is increased by the growth

of a certain amount of new tissue at the hind end of the stock, where a typical growing point developes. The ventral and lateral portions of the rim of new tissue, produce the proboscis (Pl. 5 fig. 15?) and internally, the anterior parts
(Pl 6 " (16)
of the blood and nervous systems, The proboscis thus encircles the old gut at the base of the angle which is formed as the separating constriction becomes deeper. The new tissue is laid down in such a way that from the first it has the form of a head and anterior region, and although this point has just been described as one of two growing points which are active at the line of division, histologically speaking it is not precisely similar to a terminal growing point, but is rather of the nature of an active area. The actual amount of growth taking place here as measured by cell division, does not seem to be greater than at any other point on the bud, and the increase of tissue is actually the result of the accumulation of amoeboid cells which wander into the head lobes from the more posterior segments. (This is further described in the last section of the paper). The difference is not very significant, and in any case it is difficult to imagine that ~~a~~ atypical segments such as are present in the head region, could possibly be formed by the normal method of production of new segments: the differences in the two methods of proliferation are considered later from the

point of view of "germ layers"

Behind the branchiae, in the enlarged base of the newly-formed tissue, the collar chaetae appear, and at the same time dorsally to them, a curved fold of the body wall, which is the rudiment of the thoracic membrane. A second considerably smaller bunch of chaetae develops later each side, and internally the coelomic cavities of the corresponding somites are outlined. (Pl. 2 fig. 2H). Meanwhile, the parapodia of the most anterior of the old segments have been losing their chaetae and developing fresh ones. The most anterior of these segments transformed, belong to the abdomen while they are in the stock, but are thoracic when in the bud. Such a change entails a reversal in the arrangement of the long chaetae and uncini. This interchange is beautifully illustrated in segments which happen to form the new chaetae before they lose the old ones, and so show the new long chaetae issuing from the same parapodial lobe that stills bears some of the old uncini, or vice versâ. Generally the old ones are completely lost first, in fact there is often an achaetous segment between the last old segment and the first new one, representing the intermediate stage. The membrane is spreading posteriorly at about the same rate as the transformation of segments progresses. Apart from this change of place of the chaetae and the growth of the membrane, there is no other external change involved in the transformation of abdominal into thoracic segments. The external transformation proceeds more or less segment by segment from the anterior end in sequence, hence at any one time there is a gradation of stages along the animal.

A brief summary of the condition of a typical bud at a definite age, e.g. one having its branchiae in the form of four equal, elongated, but unbranched filaments (as in Pl. 2 fig. 2C and 2H), will help to correlate the descriptions of the series of events happening in the various organs. The bud now, is at the stage of having a newly formed collar segment, and one post-collar segment: this is the newly formed tissue, as contrasted with the transformed tissue, which comprises three or four segments bearing new small chaetae, also one or two achaetous segments. Finally there is the old, unchanged posterior tip of the stock, together with any somites which may have been formed at the growing point since the bud was defined: these however are not recognisable with certainty.

In older buds, the changes already described are carried further. The branchiae elongate and develop pinnules, and opercula also when these are present on the stock, so that except for the fact that they do not bear cilia, they are precisely similar to those of the parent: the cilia do not develop until the bud is freed.

The transformation of abdominal into thoracic segments continues, probably until the total number agrees with the number in the stock. As it is a practical impossibility to collect large numbers of buds at the moment of separation, it is uncertain how accurate is this correspondence. Counts made on old buds which are still attached, have always - with one exception - shown the thorax of the bud to contain the same number, or fewer, segments that the thorax of the stock. Those with a smaller number are not significant as they are probably still growing. The one exception, showed a thorax in the stock of seven segments, and in the bud of eight; hence it is apparent that the agreement is not absolutely precise.

Segments are constantly being produced at the growing tip, so that the number of post-thoracic segments always remains somewhat greater than that of the thoracics. Finally, when the thorax is complete, the following two segments lose their chaetae and remain achaetous: first the long chaetae are lost, then the uncini, and finally the parapodial lobes. This process is not completed till after the bud is freed.

The next few somites sometimes lose their chaetae and develop new ones, but not always. This perhaps depends on the original size of the bud. If a bud begins, as it often does, with seven somites, these would all be taken over into the new thorax and transformed, but if it begins with fourteen somites, then the hind ones remain abdominal in the bud. It may be that these lose their chaetae and form new similar ones, in correspondence with the reorganisation of tissues taking place in these segments, while any segments formed after the commencement of budding, form only the one permanent set.

The distinction between old and new tissue at the ~~ex~~anterior end of the bud, is marked by the presence or absence in the interior of the body cavity, of the mesodermal masses mentioned above. These appear as a double row of opaque blocks, and it is always easy to distinguish which segment bears the first of these: this is the first old somite. The most anterior block often diminishes in size, but it never disappears entirely (see Pl. 2 fig.2). Judging by histological evidence, this is due to the fact that its cells are passing forwards into the new tissue of the head. The comparative ages of the various somites is further indicated by the sizes of the chaet~~es~~ae. The first old, transformed somite, has the longest chaetae, and in the following somites the length gradually diminish~~ing~~es until the achaetous condition is reached. Anterior to this, the collar chaetae develop, but they remain much shorter than the anterior thoracic ~~ones~~ chaetae: the first post-collar somite develops its chaetae even later, hence they are always correspondingly smaller.

At the anterior end of the bud, the collar has been growing laterally round the body, and the connection between bud and stock has become gradually narrower. Finally only a slender connecting filament remains, and this is at last ruptured by muscular contractions of the two individuals. Text Figure 3A was sketched from a bud which was watched while separation took place.

At the time of freeing, the two halves of the collar are necessarily incomplete ventrally, but by the time that the last chaetae are lost from the achaetous zone, the collar is complete across the middle line.

The details of the changes which take place in order to permit the new mouth to function, cannot be actually observed, but the general outline of events can be deduced from watching the living bud. The gut, so long as the bud is attached is a comtinuous narrow tube. The new probocis is laid down at the same time as the other head structures, and hence is ready to function at the moment of freeing. The rudiment of the proboscis is the circular groove formed by the deep constriction separating bud and stock (Pl. 2 fig. 2F and 2G). It has very thick walls, and they are so close together that a cavity is non-existent at this stage.

When the bud is freed, the internal edge of this groove joins with the old gut at the point of severance, and the whole now forms a continuous and functional structure: in text figure 3A this has already happened. At the base of the new proboscis, the old canal is replaced by new cells, and these are laid down in such a way, that when complete, a typical thoracic chamber results. The lumen enlarges at once to fill the body cavity, and in the early stages, this gives the gut a characteristic T-shape (Text fig. 3B). The new wider canal gradually encroaches on the old gut until the whole of the thoracic region has been replaced. By this time it has almost the normal appearance of a thoracic gut, though it is not yet pigmented as it is in older individuals. The swollen region of the gut in the achaetous zone is formed still later. It seems that the high columnar epithelium of the thorax is continued into this region, and afterwards as the cavity becomes distended, assumes the cubical form.

The mesoderm blocks are still conspicuous at the time of freeing, but gradually, as the wide thoracic gut is extending posteriorly, they diminish, and at last all trace of them is lost. (see Pl. 1 fig. 3).

The characteristic appearance of the gut, and the persisten~~t~~ce of the mesodermal blocks, indicate at a glance a recently freed bud (Pl. 1 fig.3.). Another less conspicuous distinguishing feature is the fact that the glandular sacs (see p. 116) are at first not pigmented. The flame like cilia can be seen in action while the bud is still attached (Pl. 2 fig. 2H), but pigment is absent at first.

External changes in the stock are slight compared with those in the bud. The anal papillae are formed, and sometimes, though not often, two or three segments are regenerated by this growing point while the bud is still attached (Pl. 2 fig. 2F). When this is so, there is a sudden transition between the old and the new tissue: the few recently added somites are smaller and narrower than the old ones, are more transparent, and have smaller chaetae. (See Table I Col.1 last 5 individuals.) After the separation of the bud, this regeneration of the posterior end continues, and for some time, the conspicuous difference between the old and the new somites persists (Pl. 1 fig.4.). The absence of the anal glandular patch at this stage, is mentioned below (P. 122)

The whole process of bud formation takes place in a short time. On one occasion a few budding individuals were isolated in a finger bowl. In twenty four hours, all the buds had separated, one, which was at the very earliest stages on the previous day, had just recently been freed.

EXTERNAL FEATURES. 4. Grouping of segments considered numerically.

In this section of the paper, the buds and stocks are regarded from the point of view of size, this being measured not in units of length, but by counting segments. It is seen later that length of body is correlated with age, though it is obvious that in an animal that buds, the two are not simply proportional. The Syllids have already been studied from this numerical aspect by MALAQUIN, ALLEN and OKADA (whose results are still unpublished) . Their results show that the position on the stock at which a bud separates is usually a fixed point - the constancy in the position of the plane - or planes - of fission being very striking,

"Non seulement chez les differents individues d'une même espèce, mais aussi chez les espèces differentes et même chez plusieurs genres" (MALAQUIN)

There are comparable records concerning the position of the plane of fission in the Oligochaetes, and these show that there is often regularity of some definite kind, though not necessarily constancy. Data giving the sizes of the buds corresponding to these variable stocks in the Oligochaetes are not ~~given~~ available, and hence a complete comparison of these with *Filograna* is not possible. This is unfortunate, as data collected from a varied series is much more likely to lead to the discovery of the factors which control the establishment of the zone of fission than a study of constant, permanently fixed, types, such as the Syllids provide.

In *Filograna* there is neither the constancy that is seen in the Syllids nor the extreme variability of some of the Oligochaetes, but a more or less controlled condition intermediate between the two. There is, in both the Oligochaetes and in *Filograna*, not only variation in different individuals, but in the same individual at different phases of its life history. (See below, page 52).

Before counting the somites, it is obvious at a glance that there is much variation in the size of the buds: by counting accurately the somites in large numbers of budding individuals, a certain amount of order is found among them.

Now that the general evolution of the form of the bud has been described, the essential features - essential from the numerical point of view - of the origin of, and the series of changes in, a budding worm, may be summarised and represented by a numerical formula. This permits tabulation of the results and so facilitates the comparison of large numbers of individuals.

The segments in the different regions of the body are counted from the anterior end, but in the majority of cases the two achaetous segments are omitted. When this is so, it is an indication that they are normal: if they happen to be abnormal, i.e. to bear parapodia or chaetae, they are inserted between brackets.

The zones are differentiated thus:

collar, rest of thorax abdomen
e.g. 1·7 14

In the case of a budding animal, the abdominal segments in front of the bud, and those included in the bud, are counted separately, the level of the head of the bud being indicated by an & sign: e.g. 1 · 7 8 & 8 (eight segments in the bud and eight in the abdomen of stock anterior to the bud). In cases in which the two sides do not correspond, the ½ sign is used, as for example a thorax with 7 parapodia on the right and 8 on the left, and a correlated inequality in the achaetous zone (2 achaetous segments on the right and 1 on the left), is formulated 1·7½ (1½) etc. Such a thorax is in process of extending itself posteriorly by means of a recommencement of the process of transformation of somites, similar to that described above (see page 29). When this happens, the first achaetous segment develops chaetae, and to compensate for this the first abdominal segment becomes achaetous. An animal which has recently added to its thorax in this manner (as indicated by the small size of the last thoracic chaetae) if formulated thus: 1.7.1. etc. Further, when the abdomen still has an obvious transition between the regenerating tip and the rest of the abdomen (see page 35), the numbers counted on the two regions are separated by a + sign.

The various kinds of individuals found, represent the different stages of the life history, and they can be arranged in the following classes.

A. Young individuals between the time of settling of the larva and the production of the first bud.
B. Budding forms with no sexual organs present:
    (a) during the first budding season.
    (b) during subsequent seasons.
C. Stocks recovering after having produced a bud.
D. Buds after freeing, still showing signs of their origin.
E. Sexual forms
F. Budding forms with sexual organs in the stock.

CLASS A.

The young individuals settle when they possess the first three segments (as counted by chaetae), and build the tube (text Fig. 4.). The next somites formed are at first abdominal, but the anterior ones become transformed into thoracic or achaetous segments, in the same way that this happens in the bud. Segments continue to be formed posteriorly until the abdominal segments number approximately twice as many as the thoracics. When this stage is reached, the posterior half of the abdomen may be separated as the first bud. Some larvae which were kept in an aquarium during one winter reached this stage in about six months: they settled and built their tubes in August, and in the following April, all were producing buds. It is a peculiar fact, that adults brought in from the sea and kept in an aquarium, always come out of their tubes in a few days and lie on the floor of the tank: but on two occasions when embryos were placed in finger-bowls, they settled down, and not only built tubes, but stayed in them indefinitely. Embryos produced during the early summer may enter at once another sexual phase (see p. 42): ORTON (1914) examined these young sexual forms and found that individuals aged 11 weeks contained fully formed eggs. He also found that buds were produced at an age of 4 weeks 2 days. The discrepancy between this figure and that given above is probably accounted for by the difference in time of year, and by the aquarium conditions.

CLASS B (a) (Table I)

The level at which the first bud appears is not constant, but there is a tendency for it to occur in such a position that the three regions, thorax, abdomen, and bud, contain all more or less the same number of segments. Such a case is expressed by the formula 1·7 7 & 7, at its very earliest stage, before there is an activity at the growing point.

CLASS C (Table II)

In the case of animals budding in the first season, recovery of segments takes place as described above (page 35).

CLASS D.

The general appearance of the buds after freeing has been described also (page 34), for purposes of this section of the paper, they are identical in their behaviour with sexually produced forms of the same size (Class A) and require no special mention.

CLASS E. (Table IV.)

The sexual individuals are hermaphrodite and the characteristic feature of this stage of the life history is the great length of the abdomen. There are commonly about 40 somites, of which the anterior are swollen and tense with a mass of genital cells, and the posterior are clear and flexible.

MALAQUIN states that the male and female glands occur in distinct somites: this is not necessarily the case. It is not uncommon to find a segment male one side and female the other. This happens sometimes in the segment lying where the male (anterior) and female (posterior) somites ~~occasionally~~ are adjacent, but in long testes (10 - 12 somites) an odd female half-somite is occasionally interpolated. Further, a particular somite may be at one time male and at another female. The two anterior sexual (i.e.abdominal) somites are always male, but the following eight or twelve may be of either sex. Individuals with long testes are seen - though not commonly, and stages of the gradual replacement of the posterior testes by ovary are found, Lastly, several times oocytes have been seen among the spermatocytes of a male gland. These were in all cases immature and apparently do not continue their development simultaneously with the sperms. During late summer, young individuals (14-20 somites, length 1·5 mm) are found containing sexual organs: these are presumably sexually pro~~vided~~ produced individuals, which at once enter another sexual phase, without passing through an intervening asexual phase. (2nd half Tab. IV) In the late autumn when the sexual season ends, there is a period of inaction until the following spring, when budding begins again.

CLASS B (b)

Just as in the buds of Class A, there is here again a tendency for the plane of fission to be situated in such a position that the number of segments before it and behind it, are more or less equal to each other (though not to the number in the thorax in this case). Hence as the abdomen in these individuals which have passed through a sexual phase is very long, it follows that the buds of this class are also abnormally long: sometimes they possess 20 initial segments. In other respects they resemble those of Class B (a) (Individuals of this type occur only in spring: they are included in Table I column II).

CLASS C.

As regards the stock of these second season buds, it appears that there is not necessarily an immediate regeneration of posterior segments. These stocks commonly possess about 20 abdominal segments, and abdomens of this length are not seen with regenerating posterior ends. On the other hand, it is very common to find them budding, dividing more or less in half, and thus producing an animal of such a type as 1·7 10 & 10.

CLASS F. (Table III)

Buds arising on abdomens which contain sexual organs, are similar in their origin and development to those of Class B: the sexual cells may occur in any or all the segments of the abdomen of the stock. They are never abundant, and, in the case of the ova at least, always remain small and immature; the sperms appear to be mature however.

The preceding facts have been summarised from tables of figures (Tables I - IV) derived from samples of colonies taken at random from all possible collecting grounds at all seasons of the year.

The counts of buds refer to the very young stages, unless otherwise stated, before there has been any proliferation of segments posteriorly at the growing point: in the exceptional cases for which older buds have been used, the age is given in terms of the branchiae on the bud head. The number of pairs of branchial lobes is given, and if the bud is sufficiently old to have the filaments pinnate, this is mentioned.

In the earliest stages of budding, it is sometimes very difficult to decide precisely where is the dividing line. In many cases there is no doubt, as the mesodermic blocks are accurately confined to the bud segments and are definitely either present or absent: in other cases, they diminish gradually, and this may introduce an error of±1 or even 2 segments.

A large number of buds conform more or less accurately to the type of formula mentioned above viz 1·7 7&7, but others tend to group themselves in the manner described as applying particularly to the buds of Class B (b). Formulae of the former type have been collected into Table I Column 1, and those of the latter, into Column 11. A third possibility is that the number of segments in the different zones may be multiples of - instead of equal to - each other; e.g. 1·7 14 & 7: such formulae are grouped into Column 111. There are still other combinations of numbers which do not seem to fall in with any devisable scheme. This is not surprising considering that there is so much variability in the behaviour of these animals, and that even in the "typical" cases, there is often not absolute accuracy in the numerical relationships, but that there may be a divergence of ±1 or 2 segments from the theoretically calculated number. The possible error in making the counts must also be considered. The aberrant cases are placed in Column IV.

TABLE 1.

Individuals of Class B. Buds (of first and subsequent seasons), arising on asexual stocks.

Column 1.

1.5	5 & 4
1.5	7 & 6
1.6	6 & 6
1.6	5 & 6
1.6	5 & 4
1.6	7 & 6
1.6	8 & 5
1.6	8 & 7
1.6	8 & 7
1.6	8 & 7
1.6	8 & 8
1.6	4 & 6
1.6	7 & 7
1;6	6 & 8
1.6	7 & 7
1.6½(1½)	8 & 8
1.6½	6½& 8
1.7	7 & 7
1.7	7 & 7
1.7	7 & 7
1.7	7 & 7
1.7	7 & 7
1.7	7 & 7
1.7	7 & 7
1.7	7 & 7
1.7	7 & 7
1.7	7 & 6
1.7	7 & 7
1.7	7 & 8
1.7	5 & 7
1.7	6 & 8
1.7	8 & 7
1.7	7 & 5
1.7	8 & 8
1.7	8 & 8
1.7	7 & 8
1.6.1(½)	6 & 6
1.7½ (1½)	8 & 8
1.7½	7½& 9

contd.

1.7.1	8 & 10	
1.8	8 & 6	
1.8	8 & 8	
1.8	8 & 9	
1.8	9 & 8	
1.8	8 & 9	
1.7.1	6 & 10	(4 branchial lobes
1.8½	9 & 11	
1.9 (1(	8 & 9	

1.7	6 + 2 & 15	(branchiae pinnate)
1.7	7 + 1 & 15	ditto
1.7	7 + 3 & 14	ditto
1.7	6 + 3 & 11	ditto
1.7	7 + 1 & 15	ditto.

TABLE 1 contd.

Column 11.

1.6	10 & 11
1.6	10 & 11
1.6	12 & 11
1.6	11 & 9
1.6	3 & 3
1.6	10 & 10
1.6.1	9 & 9
1.6.1	9 & 9
1.6.1	10 & 10
1.7	10 & 10
1.7	10 & 10
1.7	10 & 10
1.7	10 & 10
1.7	11 & 11
1.7	11 & 10
1.7	10 & 11
1.7	10 & 11
1.7	4 & 4
1.7	9 & 10
1.7	19 & 18
1.7½	12 & 11
1.7	10 & 12
1.8	10 & 10
1.8	11 & 10
1.6½	10 & 8
1.7	8 & 10
1.7	8 & 10
1.7	12 & 10
1.8	12 & 9
1.8	12 & 9
1.8	10 & 8

Column 111.

1.5	10 & 12	
1.5.1	10 & 6	
1.6	12 & 3	
1.6	17 & 13	
1.6	12 & 17	
1.6	12 & 14	
1.6	12 & 14	
1.6	12 & 14	
1.6	12 & 10	
1.6	6 + 6 & 13	
1.6	13 & 9	
1.6	11 & 9	
1.6	13 & 11	
1.6	18 & 9	
1.6	18 & 14	(branchiae pinnate)
1.6½	11 & 6	
1.6.1	15 & 26	
1.6.1	7 & 11	
1.6.1	(1) 10 & 6	
1.7	15 & 8	
1.7	14 & 6	
1.7	16 & 8	
1.7	15 & 3	
1.7	14 & 9	
1.7	14 & 15	
1.7	14 & 6	
1.7	14 & 17	
1.7	14 & 14	
1.7	15 & 7	
1.7	13 & 14	
1.7	20 & 18	
1.7	14 & 15	
1.7	16 & 19	
1.7	15 & 12	
1.7.1	17 & 8	
1.7.1	21 & 13	
1.7½	19 & 11	
1.7.1	13 & 18	
1.7	14 & 17	(branchiae pinnate)
1.8	26 & 7	ditto
1.8	15 & 9	
1.8	14 & 21	
1.8	12 & 6	
1.8	16 & 8	
1.8	16 & 6	

TABLE I (Continued)

Column IV.

1.6	8 & 4
1.6	15 & 4
1.5	8 & 5
1.7	11 & 4
1.7	6 & 4
1.7	17 & 9
1.7	13 & 9
1.7	18 & 10
1.7	15 & 3
1.7	7 & 2
1.7.1	11 & 8
1.8	7 & 5
1.8	9 & 5
1.7½	12 & 9
1.8	13 & 10
1.8	13 & 9
1.8	10 & 4
1.8	18 & 23
1.9	13 & 6
1.9	11 & 4
1.9	6 & 4
1.9	11½ & 6

TABLE II

Individuals of Class C.

Regenerating Stocks. (+ sign indicates the delimitation between old, and embryonic, (regenerated), somites.)

6

1.5	19
1.6	11
1.6	10
1.6	21
1.6	12 + 4
1.5.1	22
1.6.1	17
1.6.1	20
1.6.1	13
1.6.i	20
1.7	14 + 5
1.7	15 + 2
1.7	15 + 2
1.7	14
1.7	28
1.7	27
1.7	21 + 2
1.7	7
1.7	7 + 10
1.7	7 + 2
1.7	11 + 3
1.7	12 + 3
1.7	12 + 1
1.7	10
1.7 (0)	10
1.7	7 + 4
1.7	6 + 4
1.7.1 (1)	6 + 3
1.8	20
1.7.1	14 + 2
1.7½	15
1.7.1 (1)	16
1.8	~~4~~8 + 2
1.8	10
1.9	13
1.8.1	6 + 3

TABLE III Individual~~d~~s of Class F. Buds arising on sexual stocks.

		Bud	
1.8	17 ♂	& 13	(4 branchial lobes in bud)
1.8	8 ♀	& 17	(branchiae pinnate)
1.8	13 ♂	& 5	(very young bud)
1.7	8 ♂	& 12	(4 branchial lobes)
1.6½	6½ ♂	& 14	(4 branchial lobes)
1.6½	12 ♀	& 12	(very young bud)
1.7	1♂ 5 ♀	& 8	(very young bud)
1.7	2♂ 5 ♀ 1 asexual	& 10	(4 branchial lobes)

TABLE IV. Individuals of Class E.
(Sexual forms).

	♂	♀	Asexual	
1.7	8	0	10	
1.7	2	3	10	
1.7	2	8	15	
1.7	2	12	24	
1.7	2	15	25	
1.7	2	11	7	
1.7	2	12	25	
1.7.1	2	6	10	
1.6	2	12	20	
1.6	1	10$\frac{1}{2}$	25	~~(1.6 1 10$\frac{1}{2}$ (1$\frac{1}{2}$) 25)~~
1.5	2	10	21	
1.5$\frac{1}{2}$	18	3$\frac{11}{22}$	13$\frac{11}{22}$	
1.6	10$\frac{1}{2}$	1$\frac{1}{2}$	25	

YOUNG FORMS WHICH ATTAIN SEXUAL MATURITY
AS SOON AS THEY REACH THE ADULT FORM.

1.8	1 ♂	1 ☿	6 ♀	5	Asexual
1.7	1♂ $\frac{1}{2}$ ☿	5$\frac{1}{2}$ ♀		9	"
1.7	8$\frac{11}{22}$ ♀			11$\frac{11}{22}$	"
1.7.1	(1)		10 ♀	10	"
1.6.1	(1)		7♀	9	"
1.7	4 ♀			13	"
1.7.1	(1)		18	_ _ _ _ _ _	(with young sexual organs)
1.6.1	(1)		14	_ _ _ _ _ _	" " " "

From these results, certain conclusions may be drawn concerning the nature of the factors which determine the initiation of bud formation. In cases such as the Syllids, in which there is absolute constancy in the position on the stock at which a bud originates, no suggestion as to the reason of this can be given, whereas a variable type such as Filograna offers data from which certain facts may be deduced.

Apparently the somites are all equivalent as regards potentiality to produce the bud head and plane of fission. Determination of the position of this depends upon the parent individual considered as a whole, from the point of view of size, size of the thorax, age, and phase of the life history. Hence it follows that in any individual there are certain probable potential planes of fission, and, as an individual changes its size as a result of increase by growth or decrease by budding, so ~~that~~ the positions of these planes move, and the buds produced successively by a given stock may originate from different levels along the abdomen of that stock.

There is a more or less vague anterior limit before which a bud never arises: it is rare to find a budding individual in which the abdominal segments anterior to the bud head are fewer in number than those in the thorax, and in no case has a bud been found anterior to the fourth abdominal segment of the stock.

In spite of the repeatedly mentioned contrasts between *Filograna* and the Syllids, this general conclusion is an harmony with the observations derived from the Syllids - and further, with results obtained from regeneration experiments on other Polychaetes. For instance, the behaviour of Syllids in regeneration as described by ALLEN (1921 + 1927) illustrates the fact that every somite has a certain individuality dependent on its numerical position of the metameric series of the whole animal: in fact this character is developed to an extreme and the numerical phenomena are accurately precise. The results of BERRILL (1928) on regeneration in *Chaetopterus* show similarly that each segment regenerates according to its position on the stock.

The fact that potential planes of fission occur along the abdomen separated from each other by equal - or rhythmical - intervals is a peculiarity of *Filograna,* A parallel case of this phenomenon is given by POTTS (1913) in a description of a *Trypanosyllis*: the individual in question possessed scars marking the position of successive planes of formation of buds, in three places, these being separated by equal intervals along the abdomen of the stock.

The most outstanding impression that remains after reviewing the various kinds of ways in which buds are formed in Annelids, is that the case of *Filograna* is probably one of the most highly evolved and complex of them all. JOHNSON (1902) states that the collateral type of budding as seen in

Trypanosyllis is "unquestionably the most specialised mode of asexual reproduction, not only among the Syllidae, but among all known Annelids": it is probably equally true that Filograna is the most specialised of those which produce linear buds. This conclusion is indicated by two points: firstly by the great amount of regeneration that the tissues undergo at the time of budding, and the precocious commencement of this process, and secondly, by the fact that the head is the first portion of the bud to be differentiated. In the simpler - fission - type of budding this is not so, as for example in Aeolosoma (HÄMMERLING, 1924 (1)) and some of the Syllids; in Filograna it is probably a secondarily acquired character, evolved in order to allow the more complex structures to be completed at the same time as the less complex, and paralleled in many embryological developments.

In Oligochaetes, there is at least one genus (Lumbriculus) which on stimulation, separates its posterior end automatically. The budding in other genera of Oligochaeta may reasonably be regarded as a phenomenon of this nature, more or less specialised in so far as the head is formed at the zone of the fission at some time before rupture takes place.

The Syllids also are very liable to rupture on handling, at the points of junction between buds and stock, at any time after the first indication of the bud. In Filograna on the other hand this separation never occurs on handling, until the bud is - at least practically - fully formed.

Description of two abnormal buds

(Text fig. 5.)

Two abnormal cases of bud formation have appeared during the course of this work. The abnormality in both these is precisely the same, and the two are of the same age: hence they are almost identical. They occurred at different times - several years apart in fact - and were both found among material freshly brought in from the sea (i.e. not more than two days in the tanks). Both were fixed in Bouin's fluid and sectioned in a horizontal plane.

The peculiarity of these two lay in the fact that a bud head was being produced terminally, at the posterior tip of the stock. Four pairs of branchiae and a head lobe were present in each, hence the age correspondence to that of the normal individual figured in Pl. 2 fig. 2C. In case 1 there were 11 somites in the abdomen of the stock, and in case 2 there were 5 - containing young oocytes.

The rest of the description may be taken to apply to either individual. ~~equally~~.

The sections confirmed a fact that was suggested by the living animal, namely, that the complete bud was being produced on one half only of the stock. On one side of the animal was a normal anal papilla; on the other side was also an anal papilla, but on its mesial - or anal face, was a large outgrowth of new tissue bearing the growing branchial filaments and containing a mass of tissue attempting to form a new head. The interior of this mass contained a brain, blood spaces, a proboscis tube, and the first part of chaetal sacs, The anus is displaced from its median position by this new lobe and opens laterally.

The two peculiarities of these buds, namely, the terminal, and the unilateral, origin of the bud head, are both features very rare in Annelids. No reference to naturally occurring terminal buds can be found; but comparable biaxial heads are mentioned by HYMAN (1926) as arising among Oligochaetes in experiments on regeneration: as regards the unilateral position of the bud - though this is rare as an abnormally, yet on the other hand it is the rule in the special Syllis ramosa, and there is a comparable condition described as collateral budding in Trypanosyllis (JOHNSON 1902). There is further, a record of the production of a double head - which is probably comparable - in Typosyllis (LANGERHAMS 1881) and similar double heads are described also in Oligochaetes under experimental conditions by HYMAN (ibid).

ALIMENTARY CANAL

(See Plate 1 figures 1-4
" 4 " 9 , etc.)

The alimentary tract is a simple tubular canal, whose walls are formed by a single layer of epithelial cells: external to these cells is the peritoneum - the interval between the two being filled with blood.. Further, in the anterior half, between the mouth and the hind end of the achaetous zone, the walls are invested with a sheath of circular muscle fibres lying outside the blood sinus. The cells forming the wall of the canal are all ciliated, but by reason of certain histological changes in the character of the cells, and of changes in the size of the lumen, four anatomical regions are distinguishable.

The funnel-like mouth leads into the tubular proboscis, which extends as far back as the middle of the collar somite. Here it forms a bulbous swelling and opens into the much wider thoracic chamber - which almost fills the body cavity. The lumen enlarges even more in the achaetous zone to form the very capacious third chamber whose walls lie close against the body wall and may even distend this. On entering the abdomen, the canal narrows again suddenly to a comparatively slender tube, the intestine, which passes in a sinuous course to the anus.

The canal in the thoracic and achaetous zone is darkly pigmented, but in the abdomen is almost colourless. Very frequently the canal is empty, but at other times the large anterior chambers contain masses of structureless debris, which is kept constantly in rotation as a result of the action of the cilia.

Mitotic divisions of the ciliated cells are often seen at the anterior end of the thorax, in the neighbourhood of the lateral angles of the chamber. (Pl 4. fig 9) Between the most posterior cell of the gut and the adjacent epidermal cell (the junction being marked by the termination of the cilia), is a pair of large vesicular pear-shaped cells. (Text fig: 9e).

The cells forming the epithelium of the gut wall, are columnar in the proboscis and thoracic regions (height 30μ...), cubical in the abdominal region (height 20μ...), and still flatter in the achaetous zone (height 15-10μ). The nuclei of the cells in all cases are central and oval. The cytoplasm is more or less vacuolated and has a striated outer border. The cilia are inserted in a double row of basal granules, from which fibres converging in groups pass into the cytoplasm.

There are exceptions to the above statement that all of the cells forming the gut wall are ~~abated:~~ ciliated: a few non-ciliated cells occur in the proboscis, and in the thoracic region, these being of the nature of glandular secreting cells. In the proboscis, the glandular cells are pear-shaped and filled with a mass of darkly-staining granules, and are distributed among the ciliated cells, particularly at the base of the proboscis (Pl.4 fig.9). In the thorax, the isolated glandular cells appear polygonal in surface view, and in section are conical, with the nucleus situated in the broad basal portion: (Pl.5 fig.11) These cells are filled in some cases with granules, which stain darkly with iron haematoxylin: in other cases they are empty and collapsed, and hence hard to distinguish. They have an aperture, guarded by two small granules, leading into the gut cavity: this is the chief evidence for regarding them as secretory. There is other evidence of this fact, derived from a comparison of the contents of the cells at different phases of their activity. The other, ciliated, cells of the epithelium, are vacuolated and shew black granules, very variable in number, when fixed by any osmic acid technique. (The pyramidal cells do not blacken with osmic acid.) Individuals which have very few vacuoles, or blackened granules, in the ciliated cells (presumably starving), have densely packed pyramidal cells - while on the other hand, individuals with discharged, and collapsed, pyramidal cells have their ciliated cells filled with digestive vacuoles.

The previous authors who have studied the histology of the alimentary tract of related Annelids, are Soulier (1891) and Brasil (1904). The former finds that mucous cells occur in the oesophagus of other Serpulids, but this has not been found to be the case in Filograna. Brasil describes in detail the alimentary canal of Lagis Koreni; this is not a member of the same family as Filograna, but nevertheless it shows resemblances with Filograna in some points: for example, the appearance of the secretory cells, "cellules claviformes" is exactly comparable to that of the pyramidal cells described above, and one is figured (fig.IV) discharging into the lumen similarly.

Several attempts were made to study further the process of absorption in the gut, but unsuccessfully. The method used was as follows:- To the water in which the animals were lying, a small amount of iron saccharate (ferrum oxydatum saccharatum) was added. After a period of time varying from a few hours, to two days, individuals were fixed in Bouin's fluid to which 5% ammonium sulphide was added. It was hoped that the iron compound would by this method be stained, and thus demonstrate the process of absorption. The method was modified in several ways, but the best results obtained only succeeded in staining the masses of saccharate lying in the lumen, and did not shew a trace of the positive reaction within the cells. Unfortunately this subject could not be pursued, owing to the difficulty of obtaining, and maintaining, healthy, living material at a distance from the sea.

BODY WALL

The body wall is composed of:-

1. epithelial cells in a single layer, with the cuticle secreted by them externally, and their basement membrane internally:
2. the muscular layer - longitudinal only in some places, and longitudinal and circular, in others:
3. the central nervous system, since this is in close connection with the epidermis throughout:
4. the somatic peritoneum.

1. The epithelial cells vary greatly in form and size in different parts of the body, and cells of special function (e.g. glandular etc.) occur in places: the several varieties of these cells will be considered in order. Some of these observations have been made on living animals, aided at times by the use of intra-vitam stains, others are based on sectioned material.

CILIATED CELLS. The ciliated areas of the body wall, are in only a few cases of the nature of a true ciliated epithelium: for the most part they are merely typical cubical or columnar epithelia, in which ciliated cells are scattered. The true ciliated tracts are restricted to the branchial grooves, the dorsal surface of the tip of the head, and two transverse bands at the back of the head (Pl. 1 fig. 1, cil. b.1, and cil. b.2). These cells on the head are the largest of all the ciliated cells, being tall and slender. Their bases are branched (Pl.4 fig.9. & Pl.5 fig. 14), and they stain very darkly with Heidenhain's iron haematoxylin. On the thorax and abdomen, the ciliated cells are similar in size and appearance to the other cells of the epithelia in which they are situated: hence they are much smaller on the abdomen than on the thorax.

NOTOCHORDAL CELLS. The branchial axis is formed by clear colourless cells: these are rectangular in shape, and arranged in perfectly regular rows along the external face of the axis. At the base they become more polygonal, and finally after the union of the four filaments, are shaped like scallop shells. In surface view the cells on the axis have a diameter of 50 μ, but the larger ones at the base may reach 100 μ. These cells are very similar to those described by Giroud and Gleize Rambal (1925) in other Serpulids. They are almost transparent both in living and in fixed material, but show a delicate honeycomb in their interior. In life this is greenish: in sections it is seen to be formed by lamellae crossing the cavity, mostly parallel to the long axis of the cell. The protoplasm is small in amount and forms a border to the cell on its outer face: in this the nucleus is situated (Pl. 3 fig. 5A). The rest of the cell is filled with large, clear vacuoles, and no doubt the fluidity and turgidity of these cells gives them value as a flexible skeletal support to the axis, somewhat of the nature of a notochordal rod. These cells are referred to by DE ST. JOSEPH as mucous cells, but they do not give the reactions of mucous cells.

Other cells similar to, but not identical with these occur in other parts of the body, particularly in those parts of the body wall where movements of flexion take place. There is a broad band of them along each side of the thorax, in the region of attachment of the thoracic membrane. This provides a firm basis for the attachment of the membrane, and probably serves as a kind of hinge joint. Then also, similar cells compose the anterior wall of the uncinal lobe of the parapodia. The suggestion that these have a comparable significance, is discussed on page 80 .

There are two other patches of absolutely clear vacuolated cells. These form a pair of club-shaped areas at the junction of the lateral part of the collar with the gland shield area of the body wall. They slope obliquely ventrally and laterally, and reach the external surface both laterally at the front of the gland shield, and mesially at the base of the V-shaped angle between the collar and the proboscis wall (Pl. 5 fig. 13). The actual use of these patches is not obvious; but, as in the other cases mentioned above, their position marks a joint: in this case it is the line of union between collar and body, just where a hinge-like movement will occur when the collar muscles are in action. Perhaps the vacuoles act as a cushion to absorb the compression strain which would otherwise be exerted on the surrounding cells. The particular cells which would be subject to the strain would be the goblets of the gland shield, and this vacuolated patch may be a device to protect these.

GLANDULAR CELLS. These occur in several areas, viz., a gland shield ventrally on the collar and succeeding somite, patches on the branchiae, a patch in front of, and dorsal to, the anus, and two pairs of glandular areas of the proboscis: in addition there are in places isolated mucous cells.

The gland shields are swollen areas forming the ventro-lateral body wall from the level of the insertion of the collar (even extending on to the collar), to the hind end of the sub-oesophageal ganglion - or slightly behind this. The area extends mesially as far as the mesial edge of the ganglion, and laterally as far as the base of the collar notopodium. It is subdivided on each side into four quadrants, as the result of the passage through it of the muscle M.e.c.2 longitudinally, and the posterior nerve and the blood vessel of the collar, transversely. All these structures lie superficially immediately beneath the cuticle, and hence among the glandular cells. The gland shields are composed of goblet cells. They have long stalk-like bases which stain an intense blue with iron haematoxylin. The contents of the goblets stain bright red with Mayer's muci-carmine, and yellowish with iron haematoxylin. Hence it is concluded that this is a mucous gland. (Pl.3. fig 6B, + Pl 5 fig:14).

Isolated mucous cells are found in those regions of the epidermis which are provided with cilia. There are two lateral rows in the abdomen beneath the nerve cords, and a few laterally in the achaetous zone. The only other cells which are affected by muci-carmine, are the glandular patches on the branchiae at the bases of the pinnules (see text fig.1). These take on a pinkish tinge much paler than that of the goblet cells; however on the pinnules themselves are a few isolated cells staining a dark dull red (also among the cilia). The behaviour of food particles when being captured by the branchiae suggests that they are being captured by some sort of viscous solution, so probably these are the cells responsible for producing it.

Four patches of glandular cells, two dorsal and two ventral, occur on the proboscis (Pl 1, fig: 3 ~~text fig. 7.~~) They are composed of tall granular cells, but at present no special function has been ascertained for them. It is considered probable that they may be calciferous glands, concerned with the formation of the tube, but no evidence for this statement has been found.

The anal glandular patch is a rather loosely defined aggregate of cells situated in the dorsal body wall just anterior to the anus. It is broadest posteriorly (Pl.1 fig 3, & Pl.2 fig.2); anteriorly in tapers and becomes diffuse, gradually merging with the other epithelial cells. Single lobate cells similar in appearance to those composing the gland, occur all along the ventral surface of the abdomen. When stained intra-vitam with methylene blue, the nuclei of these cells under some conditions show a deep red colour. This gland is further described in the section on "Excretion", as it appears to function as an excretory organ.

PIGMENT. The pigmented portion of the epidermis is restricted to the head and anterior segments of the thorax. The pigment is distributed in the form of small orange-red granules, round the margin of the epithelial cells on the intercellular boundaries, immediately underneath the cuticle.

CHAETAL SACS. The chaetal sacs are of two kinds (text fig.7 & 8 & Pl.4 fig 8), one bearing long chaetae and the other uncini. The former is a deep finger-shaped sac with a bifid base, from each branch of which a bundle of bristles arises - the simple-shaped chaetae in one, and the complex in the other: at the bases of the sacs are the setigerous cells. The base of the chaeta is closely invested by the cells of the sac, but the more distal part lies freely and projects through the mouth of the sac. In the case of the uncinal lobe, the sac has lost its cavity and is reduced to a thickened ridge of tissue: in this the nuclei are embedded along the internal margin, one corresponding to each hook, but cell outlines cannot be seen with certainty. In this ridge the uncini are deeply embedded, having only their distal hooks projecting beyond the surface.

MUSCULATURE

See Pl. 3 fig 6 A + B
Pl 4 " 7 + 8
Pl 5 " 11 + 14
+ Text fig. 6.

The musculature can be observed to some extent in living animals, both by examining the movements, and by tracing the course of the fibres when a favourable individual is found. But most of this part of the subject has been worked out on serial sections.

The muscles can conveniently be grouped for purposes of description into six divisions, but these are purely arbitrary and have no functional significance.

1. Musculature of the body wall:
2. branchial musculature:
3. muscles of the thorax, collar, and thoracic membrane:
4. septal and trans-coelomic fibres:
5. chaetal muscles:
6. gut muscles.

1. In the body wall are longitudinal fibres grouped more or less into four zones - or bundles: the concentration into distinct bundles is more pronounced in the thorax than in the abdomen. In addition to these four strands, there is another ventral pair of fibres, much smaller and inconspicuous. All the fibres extend from end to end of the animal, and lie almost entirely against the basement membrane of the epidermis. The four main bundles are separated from each

other by the parapodia laterally, the mesentery dorsally, and ventrally by the two nerve cords and all that lies between them: alternatively, the facts could be described by saying that the continuous layer of muscles is interrupted by these structures. One pair of bundles therefore, is dorsal (M.l.d), and one ventral (M.l.v.): the small pair is also ventral (M.l.m. posteriorly and M.pr. anteriorly as it extends into, and along, the proboscis). The individual fibres are parallel and ribbon-like, and no structural details can be observed in them.

The only place where circular fibres can be found, is in the achaetous zone: here isolated fibres are seen (M.c.) lying against the epidermis and hence external to the longitudinal muscles and to the nerve cords. The significance of the restriction of the circular fibres to this one zone, is not obvious. As mentioned above, this region of the body generally appears more or less distended owing to the enlargement of the gut cavity within it: immediately behind it there is a sudden narrowing of the lumen, and this arrangement may cause perhaps a high pressure in the chamber anterior to it. As a result of the distension of the gut, the wall lies close against the body wall, hence contractions of these circular fibres compresses not only the gut cavity, but also the coelomic space surrounding it. It is possible that these circular muscles form a pumping mechanism to drive the blood contained in the peri-enteric sinus forwards into the branchiae to expand them, or even, that after it is driven forwards by the anti-peristaltic contractions of the gut muscles, these body muscles maintain a constant pressure to retain it there. Further, if, as was suggested, there is a high pressure of liquid in the gut, the increased resistance offered by the gut contents to compression by the circular fibres would increase proportionately the effectiveness of the contraction in compressing the intervening blood sinus.

External to the longitudinal muscles are a few transverse (i.e. incomplete circular) fibres, likewise situated externally to the longitudinal fibres. Some of these will be mentioned again in connection with the collar and chaetal muscles. A few fine strands are found also in the ventral body wall, lying close to, and external to, the nerve commissures.

There is further one special anterior transverse fibre, (M.tr.a), which differs from those previously mentioned in that it lies internal to the longitudinal muscles. It lies posterior to the brain, and anterior to the posterior ganglion of the brain (see Pl 4 Pl 6 fig. 9 14) and to the transverse bar of the blood ring. Its lateral insertions are rather vague, but it turns forwards round the sides of the brain, and is gradually lost against the external sheath of the brain near to the fibres of the internal branchial muscle. This fibre may be concerned with effecting a contraction of this section of the blood ring. As mentioned elsewhere, there are no muscles in the walls of the blood sinuses, and these particular fibres are in such close contact with the blood vessel that this explanation seems possible. Further this transverse part of the blood ring is almost always empty and collapsed in fixed animals, so much so that it is very difficult to trace often, while the lateral parts of the ring are swollen and obvious. If this explanation is correct, it is probable that the contraction of the blood ring as effected by it, serves to drive the blood round from the dorsal to the ventral vessel, rather than into the branchiae.

The one position where the muscles are not in actual contact with the body wall, is in the collar somite. Here the epidermis is raised into a ridge, and the muscles continue their straight course across the base of this. The space remaining in the ridge external to the muscles lodges the transverse duct of the glandular sac, and the muscle fibres M.ch.tr., and M.br.ext. .

Anteriorly the ventral strand subdivides into sections, and these are inserted into the branchiae, the collar, and the anterior chaetae.

2. The branchial muscles are the anterior terminations of the main longitudinal strands, (with one small addition), and

they include two distinct series.

Firstly, the dorsal muscles of the body wall on each side, pass over the brain to the antero-lateral angles of the head, and then continue into the base of the branchiae, after which they are referred to as the internal branchial muscles (M.br.int.). Here each subdivides into four, one strand supplying each axis. In the axis, it lies against the epithelium of the mesial face and sends a branch into each pinnule: in the case of operculate filaments, it spreads out and forms a lining to the cavity of the cup. (Pl 3 fig 5).

The second, external, series of fibres, is derived mainly from one of the anterior subdivisions of the ventral strand. The division occurs at the hind end of the sub-aesophageal ganglion, and the branchial section (M.br.ext.I), passes obliquely dorsally between the glandular sac and the epidermis, till it reaches the common base of the branchiae.. Here it is joined by a bundle of fibres which has arisen on the opposite side of the animal (M.br.ext.II), and which ~~These fibres~~ arise against the basement membrane of the dorsal epidermis at the back of the collar somite (Text fig.6 + Plate 4 fig 7.): the fibres from the two sides cross one another beneath the second transverse ciliated band, and then spread out broadly. Of the fibres thus separated, some join the main branchial strand, while others pass almost directly forwards underneath the brain to the tip of the head, and a very few, running close to these, continue past the tip into the proboscis. The whole mixed branchial bundle thus formed lies at first against the basement membrane of the clear axis cells which form the external wall of the filaments. Almost immediately it inserts itself between these cells, and comes to lie against the cuticle adjacent to the external nerve N.br.ext.. The muscle almost at once becomes extremely small and is soon lost altogether. As mentioned above, the mechanism which effects the straightening - or extension of the filaments, is probably a pumping of fluids - both coelomic fluid and blood - into them. The external branchial muscle probably, as a result of its ventral origin, effects a depression of the whole funnel (as in text fig. 2): the crossed fibres no doubt are responsible for the very wide opening of the funnel which is seen when the animal is fully expanded. One other small isolated strand is connected with the branchiae, and this also has a ventral origin. It accompanies the proximal part of the internal branchial nerve along the sides of the proboscis, as in Pl. 5 fig.14.

3. Of the muscles of the collar and thoracic membrane, one arises from a second, more ventral branch of the ventral longitudinal muscle. This lies beneath the sub-oesophageal ganglion as far as the level of the second collar blood vessel, then passes through the gland shield into the base of the collar, and acts as an erector apparently (M.e.c.i.) A second

erector, (M.e.c. 2) lies immediately anterior to this, arising on the sheath of the sub-oesophageal ganglion, and passing externally to the connectives into the base of the collar. A third strand, (M.e.c.3), much larger than these and lying mesially to them, takes origin dorsally at the level of the second transverse ciliated band, and passes obliquely antero-ventrally far into the collar.

At the level of the dorsal origin of the muscle are a few transverse fibres forming erectors of the collar chaetae, (M.e.ch.tr.), and the muscle M.e.c.3 in its origin surrounds these fibres (see Pl 3 fig. 6 B). The advantage of this arrangement is obvious: both these fibres are erectors, and when they contract simultaneously, the condition of tone ~~of~~ in the transverse fibres, will make the attachment for the dorso-ventral fibres ~~a much~~ firmer than it would otherwise be. ~~attachment than would the basement membrane alone~~.

The chief erector of the dorsal angles of the collar is a strand which lies immediately lateral to the one just mentioned, and which takes its origin on the sheath of the sub-oesophageal ganglion close to the origin of M.e.c.2. It passes obliquely laterally and anteriorly, against the anterior wall of the collar parapodium, and finally enters the angle of the collar and spreads out in a fan-like manner over the whole of this region (M.e.a.l.) Other small intrinsic fibres helping to stiffen the collar occur laterally, and extend a short distance into the body wall.

The reflexor - or depressor - musculature of the collar is much less elaborate. There is simply a dorso-ventral muscle (M.d.c.) arising in the head, partly in front of, and partly behind the posterior ganglion: from here it goes ventrally, and is inserted in the collar among the terminations of the various erectors. The anterior half of this muscle slopes posteriorly ventrally, and hence is imagined to act as a depessor: the posterior half however, appears to be in a transverse plane but has the ventral insertions diverging. This muscle does not seem to be sufficient to account for the reflexion of the collar round the opening of the tube as it is seen when the animal is expanded: the only other suggestion which can be made to account for this, is that the relative positions of the various strands at the base of the collar forms the basis of control of the collar movements. For instance, strands which pass into the collar near to the posterior (or external) edge where it is thick and several cells across, will tend to contract this border and so reflect the membrane (see Pl 3. fig. 6A).

All the muscle fibres which have been named here are confined to the collar and the anterior portion of the thoracic membrane: the hinder part of the membrane has no visible musculature, but it invests the parapodia so closely that it is supported by the long notopodial chaetae, and is moved by their movements ~~of them.~~

4. Lying against the parapodial walls in the thorax are certain fibres which are dorso-ventral in arrangement, but which curve dorsally so that they almost line the cavity. In continuity with these, mesially to the bases of the parapodia, are more fibres: they lie against the septa and hence are referred to as septal muscles (M.s.1,2, etc.). The strand immediately behind the collar somite, M.s.3, is rather larger than the rest, and the next one anterior to this between the collar somite and the somite of the ring vessel (M.s.2) is very greatly enlarged. It forms a broad strand extending between the side wall of the proboscis and the lateral body wall, originates dorsally behind the posterior ganglion of the brain lateral to the main dorsal strand. It runs posteriorly and ventrally down to the sub-oesophageal ganglion, and is continued backwards close to the ganglion, to be inserted in the body wall immediately behind it. The first septal muscle, M.s.1, lies at the back of the brain: it is not a complete partition but is confined to the central region. In longitudinal sections this often appears to pass actually through the brain, and is described as doing so by some authors, but this is most probably a deceptive appearance due to the curvature of the posterior face ~~force~~ of the brain at the sides of the muscle fibres (see Pl.5 fig.14).

There is still one other transverse strand, anterior to the last mentioned, and hence prostomial in position: the fibres of this pass across dorso-ventrally from the head to the branchial base, at the lateral angles of, and immediately behind, the angle which separates these (M.d-v.a. fig. 14 & 13). In appearance this resembles the septal strands, but it has not been included in the septal series as its homology is doubtful: it lies in part between the two ganglia - or nerve centres - of the brain, and if it were the case that these represented nerve centres of two fused somites, then this muscle might be the remnant of a septal fibre.

In the abdomen the septal fibres are so small that they are almost absent, but they are supplemented by oblique transcoelomic strands (M.d-v) which are inserted ventrally on the body wall at the level of the nerve cords, and dorsally, either among the fibres of the dorsal longitudinal muscle, or against the border of a parapodium. These dorso-ventral fibres are present also in the thorax, and it is probable that some of the collar muscles are the enlarged anterior members of this series.

5. The chaetal muscles include two independent sets, those of the long chaetae, and those of the uncini.

The row of uncini is moved by a series of parallel muscle fibres (M. unc.), one to each hook, arising on the anterior wall of the parapodium in a transverse line parallel to the plane of the chaetal sac, and inserted into the ridge which represents the sac. They appear both in life, and in sections, as single slender fibres, having a swelling near their insertion marking the position of the cell nucleus.

When the animal is alive, the uncini sometimes keep up a rocking motion in the direction indicated by the arrow in text fig. 7. The uncinal fibres effect the initial retraction of the uncini in this motion, but the extreme retraction of the whole sac is effected by a slender fibre (M.unc.p.) inserted on to the external and posterior margin of the uncinal lobe and arising on the hind edge of the parapodial lobe. No set of fibres exists which could be antagonistic in their action to these retractors, hence some other mechanism must exist to bring about the return movement of the uncini. The mechanism is probably produced by the passive, spring-like action of the cells of the parapodial wall. The cells on the anterior wall are large and columnar, while the posterior face is formed of extremely thin flattened cells. When the uncinal fibres are in a state of contraction, the curvature of the anterior face of the parapodium wall is increased, and the increase must exert a compression on these turgid cells. It may be that their elasticity causes sufficient resistance to this compression to cause the uncini to return to their position of rest as soon as the muscle is relaxed. Text fig. 8 shews drawings of two adjacent parapodia superimposed: one of these happened to be fixed with the uncinal fibres in a state of contraction (drawn with a dotted outline), and the other, (with a plain outline), with the fibres relaxed. The effect of the contraction of the uncinal fibres on the anterior face of the parapodium is demonstrated in this way, and the appearance supports the suggestion given above as to the significance of the histological structure. This special device for moving the uncini is the most interesting item in the muscular system. It is an extremely simple and economical arrangement, and, judging by appearances, perfectly efficient. Whether there ever was a set of uncinal fibres corresponding to the protractors of the long chaetae is unknown, but there is no trace of them in the adult animal.

The long chaetae are moved by two sets of fibres - protractors and retractors. Of the former there are 10 strands, arising round the edge of - or at the apex of - the parapodium, and inserted into the bases of the two lobes of the chaetal sac. Three of these arise dorsally in between the dorsal fibres and the basement of the epidermis (M.p.ch.1-3). A fourth is ventral and short (M.p.ch.4), arising in the region of the main longitudinal strand. Then there is an anterior strand, and a corresponding posterior strand (M.p.ch. 5 & 6) from the base, or rim, of the parapodial lobe, the posterior one of one segment arising just dorsally to the anterior one of the segment behind. There is a second anterior fibre arising on the wall of the lobe near its base, and a posterior one (dorsal), from the middle of the posterior wall of the lobe (M.p.ch. 7 & 8). There are also two long slender fibres arising at the apex of the lobe (M.p.ch. 9 & 10): the former is dorsal and arises in the angle between the anterior face of the uncinal sac and the parapodial wall, and the latter, more ventral, arises actually on the basement membrane of the cells of the sac. These ten fibres are arranged to form a cone, though not a perfectly symmetrical one.

The retractors likewise form a cone - a more symmetrical one - whose apex is formed by the mouth of the chaetal sac, and whose base by the inner rim of the parapodial lobe: (M.r.ch.). They are aided by a strand which passes from the base of the chaetal sac down to the ventral body wall at the level of the nerve cords (M.r.ch.p.). This strand in the case of the collar bristles can be traced through several somites. (Pl 3 fig 6B).

All the muscles belonging to the collar bristles are much larger than the corresponding muscles in the other somites. Owing to this fact, some fibres can be seen here which altogether escape notice in the other parapodia. However, having once found them in the large collar condition, indication of them can be observed in other places: although it is practically certain that they are repeated all along the series, this is never very conclusively demonstrable. One of these (M.e.ch.p), is the third, dorsalmost branch of the main ventral muscle. It passes up the posterior face of the collar parapodium, and is inserted on the posterior face of the chaetal sac near its mouth: hence it will effect a posterior rotation of the bristles. The second is a transverse strand (M.e.ch.tr.): it is composed of two parallel fibres and runs beneath the second transverse ciliated band at the back of the head. The two ends are inserted into the inner faces of the collar chaetal sacs, hence it serves to approximate them, and thus to raise the angles of the collar itself.

The first of these two muscles is probably antagonistic to the retractor M.r.ch.p. It is supposed that the animal moves in and out of its tube by levering itself on its chaetae - no other method would appear to be sufficiently rapid to account for the instantaneous disappearance of the creature into the tube when disturbed. If so, then the action of the muscles of the series corresponding to M.e.ch.p., would protrude the body, and the more powerful M.r.ch.p. series would effect the return into the tube.

3. The alimentary canal is invested with a sheath of circular muscle fibres between the hind end of the achaetous zone and the tip of the proboscis, lying external to the perienteric sinus. These are responsible for the anti-peristaltic contractions which sometimes pass over this region.

Although the muscles in this description have been grouped according to function, they would fit equally easily into a scheme based on homology, since they are all probably to be regarded simply as somewhat modified members of a metameric series.

The simple nature of this muscular system is shown in several points.

Firstly in the very slight development of circular fibres in the body wall: secondly by the absence of musculature in the abdominal part of the alimentary canal wall: thirdly by the absence of any special muscles in relation with the vascular system.

CENTRAL NERVOUS SYSTEM

The nervous system throughout its course is in close contact with the epidermis: it is not clearly separated off from this, and the ganglion cells lie among the bases of the epidermal cells. The fibrous cords themselves lie also among the epidermal cells in the posterior part of the abdomen, and the branchial nerves have a similar position. The two halves of the cord are widely separated and connected by commissures, so that a typical ladder-like arrangement results. The peri-oesophageal connectives are short and broad, connecting the sub-oesophageal ganglia with the postero-lateral regions of the brain.

The brain occupies almost the entire cavity of the head (see Pl. 4 & 5 fig. 9,11 etc.). The nerve cells are restricted to the periphery, and the arrangement both of these, and also of the fibres, suggests that there are two pairs of ganglia in the cerebral mass. An additional pair of ganglia, the posterior cerebral ganglia (p.c.g.), lies immediately behind the brain. It is usual for the brain of Polychaetes to show three pairs of ganglia (for bibliography of this subject see Fortuyn 1920), but in this case the third pair is separate from the other two, hence the concentration of nerve centres is less here than in such a genus as <u>Nereis</u>. In other Polychaetes, this third ganglion supplies the nuchal organ: in this case it is in very close contact with the cells of the first transverse band of cilia, but with the methods which have up to the present been employed, it is not possible to state definitely whether or not there is a fibrous connection between the two. The detailed histology of the nervous system has not been undertaken as it was considered to be too large a subject to deal with in a general paper: hence only the general anatomical features are described here.

The suboesophageal ganglia are merely the enlarged ganglia of the collar somite: they are followed by a series of comparable smaller ganglia in the other thoracic somites. In the abdomen the cords have no ganglionic swellings, they taper gradually and terminate in the anal papillae.

Cerebral nerves. There are two pairs of nerves issuing from the brain to supply the branchiae. One leaves at the antero-lateral angles of the brain (N.br.ext.) and passes into the branchial base, where it bears a ganglionic swelling (see Pl.5 fig. 11, 12 & 14). From this, branches arise to supply the tentacle and the external faces of the branchial axes. Two of these external strands pass up each axis (Plate 3 fig. 5a): at first however, the adjacent strands of neighbouring filaments are confluent, so that there are at first five, and finally eight of these strands on each side. For the first part of their course they lie against the basement membrane of the external wall of the filament, but almost at once they insert themselves between the cells and come to lie close against the cuticle. They give a branch into each pinnule.

The second branchial nerve (Pl.5 Fig. 13 & 14) leaves the brain at its hind end, ventrally and near the middle line. This nerve in the first part of its course is closely applied to the proboscis and provides the nerve supply for this. After leaving the brain, the nerve turns forwards and runs along the sides of the proboscis, giving off at intervals branches which encircle this: when close to the mouth it enters the branchial base, and almost at once divides into four. One branch enters each filament, lying on the mesial side of the axis and in the middle line: (see ~~text~~ Plate 3 fig. 5 a & b). It lies inside the epidermis against its basement membrane, and is referred to as the internal branchial nerve (N.br.int.). During its course up the axis it gives off a double series of lateral branches to the pinnules.

The fibres composing the internal branchial nerve arise in the hind part of the brain, but those which form the external nerve, though they issue from the anterior end of the brain, can be traced to a more posterior origin. They pass posteriorly through the dorsal part of the brain into the posterior cerebral ganglia. Hence in their origin as well as in their final disposition in the branchiae, they are posterior to the internal nerves.

Sections cut sagitally (and the figures of these) show further that fibres from the posterior ganglia take part in the formation of the peri-oesophageal connectives: they unite with the main bundle soon after it leaves the hind end of the cerebral mass.

Arising from the sub-oesophageal ganglia are two pairs of nerves which supply the collar and thoracic membrane. The more anterior (N.c.a.) arises at the level of union of connective and ganglion, passes forwards close to, and parallel to, the muscle M.e.c.2. It lies between the two layers of cells forming the collar (Pl.3 ~~text~~ fig.6). The more posterior (N.c.p.) arises in the same transverse plane as the commissure, and passes laterally into the membrane, lying adjacent to the posterior blood vessel of the collar, and external to the glandular sac.

The nerves arising from the posterior ganglia are extremely small, but at least one pair can be found in each somite, and traced as far as the base of the parapodium.

There are two items of special interest in connection with the nervous system.

Firstly, the incomplete incorporation of the posterior ganglionic centres with the main cerebral mass~~,~~ is a sign that there is a less degree of concentration of ganglia (i.e. of cephalisation) here, than in the free-swimming Polychaetes.

There is a comparable condition in some of the Capitellidae according to Eisig (1887): in this family it is possible to make a series demonstrating the various degrees of independence - or conversely of concentration - of the the three pairs of cerebral ganglia. At one end of the series is Dasybranchus, shewing extreme independence of ganglia, and at the other end is Capitella, ~~which~~ while intermediately are such genera as Notomastus and Mastobranchus. The condition in the latter agrees most closely with that in Filograna.

A later author (TREADWELL 1891) writing on Serpula, sees and figures similar posterior ganglia, but is very mystified by them and states that he can find no mention of these "peculiar lobes" by other authors. TREADWELL further observes that, as in the case of Filograna, these posterior ganglia contribute towards the formation of the peri-oesophageal connectives.

Secondly, the description of a double nerve supply to the branchiae is of interest, although the significance of this is not known. The fact may prove to be useful in helping to determine homologies between the various types of prostomial appendages found in Annelids. In connection with the interpretation of these two branchial nerves one point of interest suggests itself. In the text, these have been designated as the internal and external branchial nerves - the nomenclature being based on their final disposition in the filaments. It would be equally justifiable to name them ventral and dorsal respectively, on the strength of the position of their origin from the brain. This method of naming suggests that the branchial filaments have changed their orientation somewhat during the course of time, in correlation presumably, with the change in position of the mouth. In a more primitive free living condition, the mouth was probably ventral, and associated with ciliated areas, as in Aeolosoma (HÄMMERLING 1924. 1.), or ciliated palps - as in Nerilla (GOODRICH 1912).

When the animal later assumed a tubiculous habit, the mouth probably became terminal - as it is in the modern Serpulids. It is suggested therefore that the branchiae were originally ventral, oral, appendages, having their ventral faces ciliated, and that they have become terminal as a result of the development of a long, anteriorly directed proboscis: the mesial, ciliated aspects of the filaments correspond according to this view with the original ventral surfaces.

TREADWELL describes the internal and external nerves in the branchial filaments, but states that they arise by bifurcation of a single nerve each side - corresponding to the external branchial nerve of Filograna. Three nerves are described as supplying the oesophagus, one median and a pair laterally. It is suggested that on further examination one of these would probably be found to be continuous with the proximal part of the internal branchial nerve: whether the median one or the lateral pair is more likely to present this feature - cannot be stated on the basis of a comparison with the present case, as in Filograna the nerves come off the brain separately, but extremely near to the m~~o~~id-ventral line.

The majority of recent work on the central nervous system of Annelids deals with the minute structure of the cords and ganglia, the course of the nerve fibres and the grouping of the nerve cells in nerve centres. It was found to be too large a problem to carry the present investigation to such details. An attempt to apply methods of intra-vitam (methylene blue) staining to the nervous system gave no results. When whole animals were used, the stain failed to penetrate, and when the animal was opened or cut in any way, the pieces obtained were very small and they contracted and curled to such an extent that they were useless. The failure to stain the fibres was simply due to inability to handle the animal: identical methods used on other larger Serpulids gave excellent results: hence if sufficiently delicate methods of manipulation were employed, it is believed that the details of the nervous fibres would be demonstrated.

In a few cases, large solitary, unipolar nerve cells with the proximal portion of their fibres, have been noticed in the brain, situated internally to the normal border of small ganglionic cells. These may on further investigation prove to be of the nature of giant nerve cells. ASHWORTH (1909) mentions the fact that giant cells do occur as far forward as the brain in some Polychaetes, and among his examples he mentions two Sabellids, namely Spirographis and Branchiomma: it seems probable that Filograna may have to be added, to this list when the question has been finally decided. If this proves to be the case, then the hypothesis suggests itself that perhaps the extreme anterior situation of the giant cells is - as is the anterior concentration of other organs - again an adaptation to tubiculous life. When the branchiae are expanded, it is they which receive the stimuli from localities the most distant from the body of the animal - and as stated above the tips of the filaments are provided with palpocils - or sensory hairs. Since therefore the stimuli which require most rapid reaction are received anteriorly, it is understandable that giant cells are more efficient situat~~ion~~ed in the cerebral region, than in the ventral cord.

SENSE ORGANS.

The eyes (see P. 16) when seen in section, are found to be situated in the interior of the brain near its ventral side: they are composed of a hollow cup of pigment globules (Text fig 12), situated within an enlarged cell, whose nucleus lies at the foot of the cup.

The nuchal organ of Polychaetes is a structure which is extremely variable both in size and form, and its homology in Serpulids has not previously been decided with certainty. The most useful diagnosis depends on the fact that it is innervated from the third cerebral ganglion - the posterior cerebral ganglion of Filograna. As stated above, this ganglion is in close contact with the cells of the first transverse ciliated band, and though a nervous connection between the two has not been demonstrated, yet it seems probable that the band may represent the nuchal organ. According to RACOVITZA (1896), the organ "n'est que la spécialisation d'un point de la surface sensitive", and further he states that at its simplest, it has "l'état primitiv d'aire sensible". Such a description would apply to the localised specialisation of the epidermal cells as seen in Filograna. MEYER (1888) states that the unpaired terminal portion of the ciliated duct of the excretory sac is innervated by the nuchal nerves and he upholds (as does also PRUVOT, 1895) that this is the homologue of the nuchal organ. No evidence has been found for this view, and in any case the particular part of the excretory canal referred to, is the one place where it is not ciliated, and hence it lacks the most characteristic feature of a nuchal organ.

A structure which is presumed to be a variety of oral sense organ is found on the dorsal lip of the proboscis near its tip: it consists of a large clear projecting cell (one on each side of the middle line), and is supplied by a comparatively large nerve strand arising from the internal branchial nerve near its base, where it is lying against the proboscis.

The palpocils are probably sensory in function, but no special observations have been made on them.

BLOOD SYSTEM.

The blood of <u>Filograna</u> is a homogeneous fluid having a green colour, due to the presence of the pigment chlorocruorin. It is devoid of corpuscles; the cells which have been described as lying in the blood sinus, are probably cells which are crossing the cavity in order to reach the wall of the alimentary canal, where they are required to replace cells which are being discarded during the process of degeneration.

The blood lies for the most part, distributed in broad sinusold spaces: it is thus practically unconfined and cannot have any true circulatory movement. Such movement as does occur is due to pressure exerted on the sinuses, by muscular movements of the body or of other organs (e.g. the anti-peristaltic movements of the gut), and is aided no doubt by the extensibility and elasticity of the protoplasmic (non-muscular) walls of the sinuses. Fuchs (1907) and Meyer (1888) both state that most of the vessels in Serpulids are contractile, and Fuchs mentions the pulsation of terminal ampullae on them, but this is not the case in <u>Filograna</u>. The only visible fibres which are in contact with any part of the system are the circular fibres which surround the peri-enteric sinus in the thoracic and in the achaetous zones. (See also Page 72, for muscle fibre M.G.a.)

The distribution of these sinuses is identical with that of the cavities (normally only virtually cavities) which may be described as blastocoelic: hence they are entirely outside the coelom and lie in fact, between it and the adjacent organs - or tissues.

The main sinus surrounds the gut from the anus to the level of the junction between the proboscis and thoracic gut at the hind end of the collar somite. It is one uninterrupted space, crossed however at intervals by extremely thin protoplasmic strands stretching between the gut epithelium and the peritoneum. The blood in the sinuses spreads into the dorsal and ventral mesenteries between the two layers of epithelium, and generally, that which lies in the ventral mesentery is restricted to the ventralmost part of it, producing here a canal resembling a longitudinal vessel: it is however not surrounded by any wall other then the covering of peritoneum. Similarly the blood penetrates into the space between the two halves of each dissepiment, but again, instead of being distributed evenly over the available area, is confined to the superficial margin of the septum, and so appears as a ring-shaped commissure connecting the sinuses in the dorsal and ventral mesenteries. Here also there is no intrinsic wall, but only a covering of peritoneal epithelium. This epithelium is sometimes, particularly in the genital segments, produced into a backwardly pointing caecum, which is filled with blood and hangs freely in the body cavity at the base of the parapodium, ending blindly. Often also, there is a smaller swelling of the sinus on the walls of the dissepiment, producing a small pouch: this becomes covered with a mass of granular cells which are distinct from the peritoneal cells, as they are much larger and contain in their protoplasm an irregularly shaped mass having a refringent yellowish appearance (Pl. 4 fig.10.). These are probably of the nature of chloragogen cells and agree with the description of similar cell aggregates given by Winterstein (1925).

Further, there is a thin sub-epidermal sinus: this is most marked in the region of the nerve cords, and forms a distinct canal accompanying these on their internal aspects. It is doubtful whether this sub-epidermal sinus is universally present. It is in all cases so thin that it is not seen, except with the aid of special methods - as described below - and it can be seen with certainty only in those parts of the body wall where the somatic peritoneum lies close to the epidermis. Where it is widely separated by the intervention of longitudinal muscles, and the "blastocoelic" space is correspondingly enlarged, the sinus seems to be non-existent.

Anteriorly the sinuses become restricted: they are confined within definite tubular channels which may strictly be described as vessels. Their walls are extremely delicate, being formed by a layer of very flat, membranous epithelial cells, identical with those of the peritoneum covering the sinuses. No doubt these vessels are to be considered as channels which have been derived by the closure and subsequent pinching off, of grooves along the walls of the sinuses. Although the development of the vessel has not been followed, there is some evidence for this to be found in the method of formation of the anterior vessels in the bud (see below P. 105).

At the base of the proboscis the peri-enteric sinus ends, and its cavity is continued forwards in a median dorsal vessel. This bifurcates posterior to the brain, immediately beneath the first transverse ciliated band (Pl. 1 fig 1, & Pl 5 fig. 11. & Pl 4 fig 9.), and the branches at first pass outwards, then obliquely ventrally and posteriorly round the proboscis. Finally the two unite with the ventral mesenteric sinus at the back of the collar somite: a complete ring is thus formed lodged almost entirely within the pre-collar somite.

At the dorso-lateral angles of this ring a branchial vessel is given off each side: this passes above the brain into the branchial base, where it divides into four. Each branch enters a filament and sends a lateral branch into each pinnule. These, together with the main axial vessel, end blindly.

From the ring vessel two other pairs of branches are given off (Pl. 1 fig. 1) to supply the thoracic membrane. The more anterior one lies parallel with the anterior collar nerve and the muscles M.e.c.2, and enters the base of the collar: the other is more ventral and posterior, and runs transversely beneath the sub-intestinal ganglion, then turns dorsally and enters the thoracic membrane. It subdivides into several branches, some of which pass to the hind end of the membrane. The number and pattern of these is not constant: they have a sinuous course, and finally end blindly: in the membrane they lie between the two layers of cells composing it.

When any particular sinus is examined in several series of sections, it is noticed that there is great varibility in the amount of blood present in the different cases: this may be taken to demonstrate the fact that transport of the fluid does actually occur in a simple system such as this. Further, the amount of blood present in any particular region seems to vary considerably with the state of activity of the animal. In a budding animal for example, the sinuses in the posterior somites are greatly enlarged: in a similar manner too, the posterior tip of a non-budding individual, i.e. the actively growing region, often appears green from the exterior owing to the enlargement of its blood sinuses. It is chiefly, though not entirely, the peri-enteric sinus which shows this enlargement. At the level of the anus where there is a transition from ectoderm to endoderm, there is a corresponding transition between the spacious sub-endodermal sinus and the more restricted sub-epidermal sinuses. Although the change from ectoderm to endoderm is abrupt, the sinuses change their character more gradually, and there may be a quite extensive sub-epidermal sinus here, large enough to give a green tint to the tip. (Text fig. 9e. Further it seems that there occurs in the hind two or three segments an extravasation of blood from the sinuses into the coelomic cavities: the appearances of both living and sectioned animals indicates this.

The observation of the finer details of this system have been aided by the application of a common chemical test for blood pigments (i.e. peroxidases) to the living animal. The method is described by Romieu (1922), but is given by him as a method for staining the Annelid nerve cord. Romieu states that the action depends upon the presence of haemoglobin in the nerve cord; in the case of *Filograna* however the nerve cord is unaffected, but on the other hand the superficial portions of the blood system (and sometimes the deeper portions also) showed the reaction beautifully. The small size, and the transparency of the animal allow the details to be examined in the living under a high magnification, even under a 1/16" oil immersion lens.

The method employed was as follows. A solution of benzidene was obtained by sprinkling a little of the substance on sea water, and leaving for half an hour or more. The substance is very slightly soluble, but a dilute solution is what is required. The sea water is filtered and the *Filograna* immersed in it for a period varying from a quarter of an hour to two hours. They are then transferred to pure sea water, and hydrogen peroxide is added drop by drop. When small bubbles of gas appear, enough has been added: the blood now begins to show as dark blue patches. Under varying experimental conditions different portions of the blood system reacted, and by examining a large number of individuals, the whole was made out. Text figures 9 a - k were drawn with the aid of a Leitz-Okular, from animals treated in this way. The shaded areas represemt the blue-stained blood: the colour is deposited as granules or small discs, and sometimes in the larger spaces, as long needle-shaped crystals lying in bundles or stars.

The sinusoid nature of all except the anterior vessels is convincingly demonstrated, and the results confirm the conclusions drawn from other observations. But the most valuable results obtained are those concerning the sub-epidermal sinuses. These are so small that they are generally invisible in sections, and in life, though visible, are so pale that their appearance is not really conclusive. They are seen only under a high magnification (e.g. $\frac{1}{12}$" obj.), and their pale green colour might be only an optical effect; but when stained Prussian blue, there is no doubt as to their presence or their nature.

The ventral mesenteric vessel and the commissural vessels are generally seen, and the ventral portion of the anterior ring (text fig. 9a.): the dorsal part being less superficial does not stain well, except in buds. The sub-epidermal sinus is seen in patches: one squarish patch constantly appears on the anterior third of the thorax dorsally, Text fig. 9c and, (not quite so constantly), another on the hind thoracic and achaetous zone. There is never a trace of blue in the one place where the sinus is enlarged and one would therefore expect it, namely along the neural sinuses: this may be due to the fact that the nerve cord itself makes a thick impenetrable ridge in the body wall, which the reagents do not pass through.

The appearance of the sub-epidermal sinus varies somewhat. Sometimes it is a broad expanse of blue: at other times clear transparent patches are left standing out against a blue background (text fig. 9c.). Such spaces correspond exactly in size, position, and shape, to one or other of the types of epidermal cells observed under other conditions: very frequently the form of the spaces coincides with that of the clear lobed cells filled with globules as described in the paragraph on the anal glandular patch. The appearance suggests that these large swollen cells obliterate the cavity of the sinus, and so are indicated as colourless areas. In still other places, the blue is reduced to a mere honeycomb, precisely similar to the pale green network which is seen in a normal untreated animal. The size of the mesh of the honeycomb varies in different parts of the body, and is evidently to be regarded as outlining the epithelial cells, as the size varies in correspondence with the variation of these cells. (Text fig. 9d).

Sometines the peri-enteric sinus is stained also, but this is easily distinguished from the superficial sinus by fo~~r~~cussing the microscope.

The appearance of the main mesenteric "vessels" is interesting too, as it emphasises the fact that they are not simple tubular canals, but open spaces. The median strand of blood fades away laterally quite irregularly, generally in a series of pointed projections which may be regarded as inter-cellular processes, and often clear cellular areas are left unstained in the middle of the vessel, as described above as happening in the expanses of the sub-epidermal sinuses. (Text fig. 9b) shows a portion of the central sinus in which one half is fairly full of blood, and the other much less full, and showing the honeycomb form rather than the typical canal.

The communication between the sub-epidermal sinuses and the larger sinusoid vessels is demonstrated by this method much more plainly than by the use of serial sections. The condition ~~in the case of~~ of the communication between the mesenteric vessels & the sub epidermal sinuses has just been described: another case is provided in the anal region. Here, as in text fig. 9e & f. the continuity between the two is obvious. Further than that, a similar condition is exhibited in the developing vessels of the head of a bud. Text figure 9 g & h show the branchial filaments of a young bud; the branching of the main branchial vessels is shown and the passage of the branches into the bases of the branchiae. Here however instead of the simple finger-shaped blind terminations that are seen in older buds or adults, the vessel fades away among the blood of the sub-epidermal region. This, together with the appearance of figure 9 k, show that the blood vessels of the bud are formed by the restriction and enclosure of the wider sinusoid expanses of the stock. Text figure 9 ~~h~~K shows the formation of the anterior ring vessel of the bud. The median vessel of the stock is apparently being obliterated at the point of bifurcation. Similarly in the branchiae, it seems that an enlarged portion of the sub-epidermal sinus becomes separated off as the vessel. (The course of events in the larva hasnot been followed).

This type of blood system is practically the simplest conceivable for an Annelid: whether it is equally primitive is not so easy to say. The question of the probable origin of, and the evolution of, the blood system in Annelids has been discussed fully by STEPHENSON (1913), and according to his theory, the spacious peri-enteric sinuses of Serpulids have been derived secondarily from a network of vessels and sinuses such as occur in the genus <u>Aeolosoma,</u> by spreading and fusion of the separate lacunae. LANG (1904) on the other hand considers that the primitive Annelid possessed a system almost identical with that just described, formed of a gut sinus lying between the gut wall and the peritoneum, with septal and mesenteric sinuses communicating with it. EUGEN LEE (1912) supports LANG'S theory entirely, and emphasises the fact that this theory of the origin of the blood spaces in Annelids accounts for the fact that the vessels have no intrinsic epithelial lining.

The complete absence of any trace of walls (apart from the peritoneum) is quite certain in the case of <u>Filograna</u>. The various authors writing on this subject are not unanimous in deciding whether the blood vessels of Annelids have, or have not, an endothelial lining. MEYER (1901) stated that the presence of an intima in the vessels of Annelids had not been proved, and that he definitely thought it was absent. The most recent paper (FEDERIGHI 1928) states that some of the vessels do possess an endothelium and that some do not. In support of the negative statement, DYRSSEN (1912) states definitely that in Cirratulus there is no vaso-endothelium, but that, "die innere Gefässmembran nur eine Fortsetzung der Grundmembran des dissepimentes darstellt".

~~This~~ LANG'S theory is supported and extended by DE BEAUCHAMP

(1911). According to this author the cavity of the blood system may be considered as a blastocoel. LANG denies this, in spite of the fact that his figures and descriptions emphasise the similarity in the distribution of the two. DE BEAUCHAMP defines a blastocoel as any cavity which separates any two of the primary cell layers, as distinguished from cavities which are comprised within one of them. Hence he considers that the cavity of the blood system is theoretically a blastocoelic space "même si elle s'oblitère avant de se reformer au cours du développement". The exact correspondence between the two series of spaces in the adult Filograna strongly supports this view, and a diagram which DE BEAUCHAMP gives (fig.7) as a schematic representation of a primitive condition, could be taken to apply to Filograna. Also, the embryological evidence of GIARD (1876) working on Salamacina, and of SELENSKY (1882) on Psygmobranchus, helps to confirm the blastocoelic hypothesis, for they describe the blood cavity as arising by a separation of the already adjacent epithelia of the gut and of the splanchnic peritoneum.

The blood fluid is distributed in Filograna in a manner similar to that in which it is distributed in forms provided with vessels and capillaries, but whether the absence of these is a case of primitive or of secondarily acquired simplicity, it is difficult to say.

Concerning the origin of the blood fluid itself, STEPHENSON regards it as an accumulation of fluid diffused through the gut walls from the enteron and containing dissolved in it, food substances, mineral matters, and oxygen. LANG, LEE, and VEJDOVSKY (1905), are all agreed in assuming that the blood was primarily a nutritive fluid, and that the perienteric spaces are the most primitive portions of the system: this last point does not hold if the blastocoelic hypothesis is correct.

In the most primitive Annelids there was probably a gut surrounded with coelomic sacs, and hence possessing a double wall. The cavity between the two layers of the wall is theoretically blastocoel, and in that case, if the cavity is still existent at that stage of development - or evolution - at which blood first appears, would already be filled with some kind of fluid. Further, this fluid would from the first be widely distributed, and of the nature of a ready-made medium of communication between the cells of the body. If dissolved food matters were secreted into it from the gut, they would diffuse by simple physical laws throughout its whole extent, and the diffusion would be aided by movements.

The difference between this view and those quoted above is that the latter regard the blood system as something arising de novo, for nutritive purposes primarily, whereas the former assumes rather the adaption of a pre-existing body fluid, and takes into account both the adaptation to food transport, and to respiration.

It is deduced from most of the authors mentioned above, that the formation of external respiratory organs was something superimposed on the blood system after it had acquired its last formed portion - namely, the peripheral; (this is presuming that respiration was a surface activity). It is a difficulty to imagine a second function being developed after the first (i.e. food carrying) was so well established, and if it be assumed that the blastocoelic fluid is the original basis of the blood, the difficulty does not arise. For at the very earliest stages, the peripheral parts are developed to the same extent as the enteric, and the two functionscan be imagined to have evolved arise simultaneously.

The suggestion that the blood is derived from the blastocoelic fluid, accords with the theories of Macallum concerning body fluids. He assumes (1926) that the blood of primitive animals was merely a duplication, chemically and physically, of the external medium in which these animals lived, namely, the primitive sea water. This is imagined to have passed by some means, into the internal cavities of the creatures, and obviously, if this were so, the water which passed in would find itself in the blastocoel.

According to this hypothesis, the vessels of other less simple Annelids are imagined to be derived by restriction of the sinusoid spaces to circular channels, as happens anteriorly in Filograna. The precise origin of the complex thick walls of these cannot be guessed from the particular genus under discussion. However a hint is supplied which may help to account for the difference in musculature between the dorsal and the ventral vessels. Filograna agrees with the general rule laid down for Annelids, that the differentiation of the ventral vessel is in advance of that of the dorsal. The ventral one shows a fairly precise circular outline, whereas the dorsal is more in the nature of a vague swelling spreading all over the mesentery: further, the ventral is more detached from the gut, and hence is removed from contact with the gut muscle. It is concluded from this, that if a vessel originates in the mesentery near its external (dorsal or ventral) attachment, or if it originates in the gut, but separates from this before the musculature is developed, then the vessel would be expected to be non-muscular: on the other hand if it does not become separated till after the gut muscle are formed, then, it is probable that it would retain the muscular coat., as it does in the dorsal vessel of most Annelids.

This particular blood system, with its anterior concentration of vessels, is adapted to the tubicolous habit of the animal. The blood is forced into the so-called branchiae by the action of the thoracic gut muscle. Anti-peristaltic waves are seen to pass over this region, and the effect of these is probably reinforced by a simultaneous contraction of the circular muscles which are present in the body wall in the achaetous zone. The expansion of the branchiae thus effected provides the mechanism for the capture of food.

The theoretical importance attached to the account of the blood system seens at first sight inordinately great compared with the amount of data given. Nevertheless, such data as there are - particularly those derived from the finer details of the blood system - are of a critical nature. They happen to fill a gap in the already established "blastocoelic" hypothesis, to provide in fact exactly the conditions that are required by this hypothesis for an initial type, as mentioned above (p. 107) *Filograna* takes therefore a place in the evidence upon which the theory is based, but has not in any way modified it.

The method employed of staining the blood fluid in the finer channels, is one which is capable of wide application.

In the hands of the chemist, the present rather crude and empirical method could no doubt be improved and made more specific. The reaction in the form in which it has been applied, is simply one of the several which are in use for the detection of peroxydases, but the author is convinced that the substance showing the positive reaction in *Filograna* was identical with, and confined to, the fluid contents of the blood vessels. No attempt was made to preserve these benzidene preparations, and it is not known whether this is possible or not.

This method is invaluable for the investigation of small animals, and has also been successfully used on larger Polychaetes e.g.(Terebellids and Sabellids), and Oligochaetes (*Lumbricus*): if freshly killed, or narcotised animals are used, the finer reticula on the various organs are beautifully demonstrated. It has also been used on *Hirudo* and might perhaps prove to be a useful supplementary method to apply to other leeches. Although the vessels of leeches are sufficiently large to inject, the chemical method would have the advantage over the injection methods, that there is no danger of rupturing the walls of the finer vessels in its application. If the method were further refined, it might assist in solving the problems of blood system of Hirudinea)

COELOMIC CAVITIES.

The coelom in the abdominal region is a fairly spacious cavity - except at the time when the genital products are mature, but in the thorax it is greatly reduced as a result of the enlargement of the gut. It is divided into chambers from the head ~~of~~ to the anus, longitudinally by the dorsal and ventral mesenteries supporting the gut, and transversely by the dissepiments between the segments. In the abdomen therefore, the cavities are simple rings. In the thorax, though the cavity is reduced, it is still obvious, but in the achaetous zone the walls of the gut are pressed against the body walls so that the coelom is almost obliterated: however the septa can still be distinguished (Pl. 2 fig.2h).

The coelomic epithelium is extremely thin, but is rendered conspicuous by the fact that there are blood sinuses separating the two halves of the double mesenteries, and underlying the splanchnic layer. The epithelium is membranous and bears at intervals small swellings, which indicate the position of the cell nuclei. In the sexual somites at the time of maturity, the cells lining the tubular genital duct, and those of the epithelium round its inner opening, become ciliated.

The most anterior somites are somewhat modified in size and form. The collar cavity is larger than any other. It surrounds the gut where this is at its narrowest, i.e. in the proboscis region; further, it possesses a pouch-like prolongation extending backwards between the gut and the coelomic cavities of the one or two succeeding somites, and lodging the collar chaetae when these are retracted (Pl.5 fig 12). The somite anterior to this, the pre-collar somite, lodges the blood ring and the branchial vessels. A dorso-lateral outgrowth on each side enters the branchial base (Pl.5 fig. 12), and subdivides to send a branch up each of the four filaments: these in turn send branches into the pinnules. These branches lodge a precisely similar system of blood vessels, which arise from the lateral angles of the collar ring. There is another outgrowth from the pre-collar cavity - the nephrostomial tube - for a description of which, see the section on "Excretory Organs ".

GLANDULAR SAC AND EXCRETION.

Filograna agrees with other tubicolous Annelids in having its Excretory organs confined to the anterior somites: ~~one pair of excretory organs~~ (MEISENHEIMER 1909): in the more posterior segments, the only communications which can be found between the coelom and the exterior are the ciliated gonoducts in the genital segments. There is one pair of anterior excretory organs, in the form of a pair of ciliated tubes, whose inner ends are swollen into sac-like pigmented bodies - the glandular sacs - and whose distal ends unite to form a common terminal duct. (See Pl.1 fig.1).

These sacs lie at the sides of the collar somite against the lateral body wall, dorsal to the nerve ganglion. At its anterior end the sac narrows to form a tubular duct, which throughout its course lies close against the dorsal epidermis. It bends inwards almost at once at a right angle, and when near the middle line, turns forwards by another right angle. At the back of the head the tubes from the two sides unite and a common median duct continues forward dorsal to the brain, to the tip of the prostomium, where it opens on a pore situated in a small median papilla and guarded by several large pear-shaper cells: (Pl. 4 fig.9). The organs are said to be the persistent protonephridia of the larva by MEYER (1888) and MALAQUIN (1908). The walls of the duct (which is inter-cellular) as seen in section are not of equal thickness all round: they are thick on the side adjacent to the coelmic cavity and thin externally (Pl. 4 fig. 7.)

Both the sac and the duct, with the exception of the common terminal part, have their cells filled with greenish granules. Also, they both are ciliated, the cilia being so long in the duct, that they appear in the living animal to form a flame following the bends of the tube, and reaching almost to the pore. The beating of this flame keeps the granules constantly vibrating.

The so-called sac is not a closed vesicle, but communicates through a pore in its outer wall with the coelom of the pre-collar somite (text fig.11). It agrees thus with the nephridia of other Serpulids, as described by MEYER (1889) and LEE (1912), although in the case of Filograna neither LEE nor MALAQUIN (1908) could find the aperture of the sac. The hind wall of this pre-collar somite is produced into a long tubular - or funnel-shaped - diverticulum, which inserts itself between the sub-oesophageal ganglion and the glandular sac (Pl. 5 fig. 11 and 12), and which lies just within the lateral body wall in the region of the muscle M.br.ext.l. The pore of the glandular sac opens into the base of this tube, hence it has the characters of an open nephrostome, being however somewhat peculiar in the disposition of the cilia connected with it. At the hind end of the sac is a larger posterior cel from which a group of very large cilia ar~~r~~ise. These pass through the pore, beat up the nephrostomial tube towards its mouth, and extend into and across the main cavity of the pre-collar somite; the length of this flame-like structure is 200μ (.2mm.). The undulating, flickering motion of these cilia can with a little care be distinguished from the similar flame-like movements of the cilia within the duct of the glandular sac. Both are obvious when the animal is slightly compressed under a coverslip and viewed from the dorsal side. The two movements are very close together, but the nephrostomial flame does not follow round the acute angles of the pigmented duct as the flame inside the duct naturally must, but takes a straighter course. Other small flickerings are seen in this same neighbourhood, produced by cilia on the walls of the pre-collar cavity main chamber; one of these beats just externally to - and parallel to - the base of the branchial vessel (Pl.1 fig.1). As there are no other such cilia in any other somite, these may have some relationship to the nephrostome.

The several parts of this organ can be homologised with the corresponding parts of the various types of nephridium found in other Polychaetes. The dorsal, median aperture remains entirely anomalous, but the rest of the organ is not so abnormal as at first appears.

In several types of nephridium, e.g. some Nereids (GOODRICH 1900), there are found tufts of cilia arising on and around the nephrostome (whether closed or open), and beating into the coelom: structures with comparable appearance and function are described by MEISENHEIMER (1909) in other phyla. Beginning with a nephridium of such a form as this, the _Filograna_ type may be deduced by imagining firstly, the loss of the pre-septal portion of the funnel, secondly the depression of the part of the septum to which the nephrostome is attached, into first a pit and then a finger-shaper diverticulum, and lastly the transference of the coelomic cilia from the margin of the nephrostome to a position just within the tube itself. There results then, with no very fundamental change, a structure identical with that found in _Filograna_.

There are other cases described by GOODRICH (1900 &) (1912) in the Syllids, in _Nerilla_ and in _Polygordins_, in which very long cilia arises within the nephrostom~~e~~ial ~~in~~ aperture, but it is stated that the cilia arise on the lip of the funnel and beat down its lumen: this however is the reverse of what obtains in <u>Filograna</u>.

The ciliated duct is lodged in its proximal part, in the transverse ridge which crosses the collar somite dorsally, and it is parallel to - anterior to - the transverse muscle band M.e.ch.tr.. It crosses the dorsal longitudinal muscle fibres externally to them, and the next part crosses the blood ring, likewise externally to it (Pl 3 fig.6).

The nature of the green granules has not been determined: they are resistent to reagents, and stain densely with haematoxylin stains or osmic acid. With a hope of determining their nature, some chemical tests for uric acid were performed on the living animal. These gave negative results as far as the glandular sacs were concerned, but as they gave evidence of the presence of excretory substances in other parts of the body, they may be described here.

The tests used were, firstly, the murexide reaction, and secondly a modification of the Benedict method for detecting small quantities of uric acid. In the first case, the method used was that described in Howland (1924), namely the addition of strong nitric acid (with or without previous treatment with 50% alcohol) followed by evaporation to complete dryness.

This last step is not so drastic here as in the case of Howland's <u>Paramaecia</u>, as the form of the animal is still distinguishable on the slide even after drying. **Dilute** ammonia was then added, but there was no positive reaction in any part of the body.

Hence the more delicate Folin-Trimble method was adopted. Weaker solutions than those given by Folin-Trimble (1924) were used and the treatment was as follows. Some animals were placed in a measured volume of a solution of 5% sodium containing 2cc. of concentrated ammonia per litre. A quarter of this volume of arsenic phosphoric tungstic acid was added, and the tube placed in boiling water for three minutes. Then it was removed and cooled in a beaker of water, after which the animals were examined under the microscope. There was no reaction from the glandular sac, but the granular cells lying in the epithelium in the region of the anus, were coloured blue. On examining with a high magnification ($\frac{1}{12}$" obj:) each granule in the cells was seen to be tinted blue. This reaction indicates that these cells are excretory in nature. The negative reaction of the glandular sacs may be due to the insufficient penetration of the fluids into the interior of the animal, or, assuming that they are excretory, to the fact, that the excretion is in the form of some substance other than uric acid. The deposition of and gradual accumulation of pigment in both these glands (as mentioned above - P 35 favours the view that they are excretory in function. In a recently budded individual, the sacs at their first appearance are colourless, and they become pigmented gradually. Further, in a stock which has lost its hind end in a bud and is regenerating another, the anal gland is absent at first, but grows in size, and increases in opacity as the tissues become older. On the other hand, the bud, which carries away the original hind end of the stock, and which haspassed through a period of particularly great activity, has an abnormally large anal patch.

In preparing the sections for this part of the work, a variety of histological techniques were tried: two, however, proved to be the most successful, and were almost entirely adhered to: these were Flemming-without-acetic followed by safranin, and Bouin (with modifications) followed by H~~e~~aidenhain's iron haematoxylin. The modifications of Bouin's fluid which gave the best cytological prepa~~r~~tations were, firstly Ezra Allen's chromic-urea-Bouin, secondly Duboscq-Russel and Duboscq-Brasil, and third aqueous Bouin. Other methods employed gave less satisfactory nuclear preparations, but proved useful in studying the anatomy and histology of the organs of the adult: these other techniques included Mann-Kopsch, Champy-Kull, Cajal, and H~~e~~aidenhains Susa fixative followed by H~~e~~aidenhains Azan stain.

HISTOLOGY OF THE BUDDING.

The internal and histological changes which accompany the development of the external form of the bud have been partially described by MALAQUIN in a series of papers dating from 1895 to 1925; the last of these (1925.(2)), unfortunately escaped the notice of the author until the particular point with which it deals - namely, the origin of the blastogenic cells - had been already worked out. The results obtained agree in the fundamenta points with those of MALAQUIN, but differ in some details from them: further, they include many observations of cytological features not mentioned by MALAQUIN. The following description therefore, will overlap somewhat that given by MALAQUIN, but it is necessary to repeat these observations here, in order to mak the later part of the subject intelligible. (Reference is alway made to MALAQUIN when his results are repeated.)

As a result of the internal changes which take place, the new individual when quite complete, is to be regarded not merel as a fragment of the stock which has been cut off and has regenerated a new head, but rather as a new, young individual - inasmuch as its tissues are all young and newly differentiated.

As mentioned before (p. 25), the head, collar, and next succeeding somite, are formed by the growth of a lobe of new tissue, and the other somites by the transformation of the posterior segments of the stock.

There are two points of interest concerning this anterior proliferation of new tissue. In the first place, since the alimentary canal is continuous throughout the stock and bud until the time of freeing, the connection between the two individuals is such as might be described as a mouth-to-anus attachment: this is not strictly true as the attachment is behind the mouth of the bud, but such a phrase emphasises the fact that if there is this particular type of connection, then all the pre-oral somites (at least) must be proliferated anterior to the plane of fission. The second fact which is suggested by these observations, is that the few anterior somites are atypical in form (i.e. non-annular), and it is difficult to imagine how they could be formed by transformation of old abdominal segments. It is obvious however that neither of these remarks explain why there is this special method of production of anterior somites, for the number of segments concerned in it is greater than is actually required to satisfy either of the two conditions.

The second point of interest concerns the manner in which the position of insertion of the new tissue and of the plane fission are related to the septa and somites of the stock. Th former is located immediately behind a septum, is therefore post-septal, and the latter is inter-septal. Plate 6 fig. 16 demonstrates this fact quite clearly. Behind the last septum of the stock (s. n.), there is firstly the newly-formed hind end of the anterior individual, then the bud head and the two setigerous bud segments, and finally the old and partially transformed segment with its mass of phagocytes, then the next septum (s. n+1). These two septa are originally adjacent: the newly-formed septa reach from the body wall to the new proboscis only, hence the old ones which stretch across the coelom and are attached to the old gut, are distinguishable.

The transformation of tissues which takes place in the bud segments is of so complete a nature that finally there is not much more connection between the bud and its original segments than there is between an embryo and its parent, except the topographical position, for the tissues of these segments are entirely histolysed and replaced by embryonic undifferentiated cells. There seems to be considerable variation in the amount of time taken for the completion of this process. In some cases it progresses gradually, so that some part of each tissue retains its histological character while the rest is in a condition of disintegration. In other cases there is a much more sudden and drastic change, during which the bud is represented by a structureless mass contained within the covering of epidermis.

This process of rejuvenation of tissues extends into the stock, but in this region the disintegration at any one time is only partial, in fact there is probably never complete replacement here as there is in the bud. The histolysis in the stock is often confined to the abdomen but sometimes extends into the thorax, and has been seen even in the brain. It happened many times during the course of this work that sections of individuals showing this phenomenon were discarded, as they were thought to have been injured during fixation. However later, the condition was recognised in living animals and discovered to be normal: the histolysis of stock tissues is seen Pl. 5 fig. 15, also in the micro-photographs. In the stock, the degeneration is accompanied by the appearance of large, orange-yellow fat globules in the coelomic cavity and in the gut; buds never contain similar globules. It is not certain whether these are of the nature of food reserves, or are simply a useless product of degeneration, but judging from their presence in the gut cavity, and from the fact that they are probably discharged with the faeces, the latter interpretation seems to be indicated. In the bud on the other hand, the material obtained by the disintegration of the tissues is utilised, at least partially, as food for the growing cells. Another similar contrast between stock and bud is provided in the fact that although in the latter the material contained in the phagocytes is all retained and utilised, in the stock there is not infrequently a rupture of the body wall, through which some of the disintegrating masses are discharged.

The disintegration may be either intra- or extra- cellular. In the case of the gut, the ciliated cells are discharged into the lumen either singly or in groups, and finally are passed out at the anus. The other tissues are all attacked by phagocytes, though at the time of greatest activity, large masses of muscle are seen to be breaking up independently; but even these appear to be dealt with by the phagocytes later.

The contents of the phagocytes are preserved (at least partially) by fixation in Bouin's fluid (whether aqueous or alcoholic), and stain after this very darkly with iron haematoxylin: they are blackened by fixation in fluids containing osmic acid. An endeavour was made to determine more accurately the nature of this substance by the application of methods of intra-vitam staining. On treatment with Sudan III (LORRAIN SMITH: 1907) the mesodermic masses in the bud assumed a bright orange colour. Nile blue sulphate was next tried (LORRAIN SMITH 1906), and it was found that this penetrated only very slightly into the interior of the animal and gave no results: to overcome this difficulty the body wall was ruptured, and then it was seen that the globules in the cells which were extruded became greenish immediately: as these are originally orange in colour, this result is taken to indicate that the blue component of the stain was absorbed: hence it is concluded that fatty acid is present in the globules.

The phagocytes, throughout most of the process remain extra-coelomic, and lie in the cavity which separates epidermis and somatic peritoneum - normally occupied by the muscles only. A few however enter the coelom and are found lying against the masses of chloragogen cells on the mesenteries, possibly discharging their contents into the chloragogen cells. The presence of such masses of phagocytes enlarges the extra-coelomic cavity, and reduces the coelom to a small + - shaped space, in the centre of which the gut is suspended: (Pl 2 fig:2~~8~~6) shows a bud as it appears when focussed deeply below the surface: the reduction of the coelom is evident here. The opaque blocks which are described above.(p. 26), and which are seen in this, and all the other figures, are merely these masses of histolysing tissues. They persist in the bud after it has been freed (Pl 1 fig.3), and there is evidence that at least some of their contents are used as nutriment by the actively growing cells, for the characteristic globules are found among embryonic cells in places in which they cannot have arisen originally. For instance, they are found in the bud head, and there never has been any phagocytosis in this region, as it is an outgrowth of new tissue. The cells forming it - undifferentiated cells - wander in from the segment behind, and some at least of them, arrive laden with the globules. Plate 6 fig. 17 shows the head of a bud fixed in Flemming-without-acetic, and the blackened globules are conspicuous in this, among the differentiating tissues. It is only in such new tissue that it is possible to demonstrate the utilisation of this nutritive matter, but such a fact seems to justify the assumption that the gradual disappearance of it in the freed bud is due to absorption of this kind, rather than to excretion.

MALAQUIN mentions the phagocytosis of the tissues and thei replacement by undifferentiated cells. He comes to the conclusion that the cells in question are derived by proliferation of the gametocytes. He has found the two primordial germ cells in the larva, and states that the cells derived by the repeated divisions of these "seront ensuite distribués dans les métamères, où ils deviennent les gonocytes extra-coelomiques .. ..leur ensemble constitue une lignée germinale autonome dans l'individue, et continue d'une génération à l'autre". (1925 1). Further, in his next paper (1925 2) he states that "lorsque, tout au début de la reproduction asexuée, les gonocytes entrent en mitose active dans un ou plusieurs métamères, les cellules qui en proviennent prolifèrent au sein du tissu mesenchymateux nutritif, sous l'endothelium coelomique .... Les éléments ainsi formés........ sont les histoblastes (ou néoblastes) migrateurs qui vont édifier less tissus nouveaux, musculaire, épithelial, etc. ."

This double row of special cells is always found in the ventral body wall. The cells are in close contact with the nerve cords, lying practically superimposed, but rather mesially to them: they are against the basement membrane of the epidermal cells and hence are covered by the peritoneum: they extend from the anal papilla into the thorax. The present author does not agree with the cytological description given of them, and instead of saying that they are gametocytes which on occasion produce tissue cells, would prefer to summarise the facts by saying that these are the only cells in the body which retain the potentiality of active proliferation.

When series of sections are examined, whatever the phase of the life history, these cells are the only ones which are ever seen to be actively dividing. In the genital somites of sexual individuals, a group of these cells situated just behind the posterior face of the anterior septum produces the sex cells; these, sooner or later pass through the peritoneum and come to lie in the coelom. In ~~The~~ budding individuals, these same cells divide actively and apparently produce both the phagocytes, and the "Ersatzzellen", or neoblasts. Also, they are responsible for the formation of the masses of chloragogen cells on the mesenteries: these enlarge at times of great activity by the addition of cells from the ventral proliferating area. The same small cells are responsible for producing all o the new tissue at the terminal growing point, details of which are given below. MALAQUIN describes the presence in the larva of two large mesoderm mother cells, distinct from the two primordial genital cells, but does not mention any further history, or divisions of these. In the full grown budding animals that have been examined in the course of this piece of work the two cannot be distinguished: there are two groups of large cells with nuclei resembling those of the mesoderm mother cells, but these are inactive and are themselves derived from th neoblasts.

The embryonic cells have already been referred to as neoblasts, and they are regarded as homologous with the cells of the same name in Oligochaetes: they have relatively the same position in the body, and the same function. The apparent difference in their position in the Oligochaetes is probably due to the fact that with the approximation of the two nerve cords, the two intervening rows of neoblasts have been brought to a median position. The presence of neoblasts in Polychaetes is denied by Hämmerling (1924) in the summary which he gives of the literature on this subject, but the homology is accepted by MALAQUIN.

POTTS mentions, in his description of *Trypanosyllis* (1913) the presence in the cushion of proliferating tissue of leucocytes and of "small nuclei with small scattered chromatin granules (mes.n.)." He also described cells whose nuclei are large and contain a large nucleolus, and he states that "I am inclined to think that these wandering cells undergo a nuclear change, and become the actively dividing cells of the mesoblast". Judging by his figures, the previously mentioned cells with scattered chromatin granules resemble more in appearance the neoblasts of Filograna, but there may be no histological similarity between the two.

The neoblasts are very strikingly different from all other cells, and hence are easily recognised. The nuclei are large in comparison with the size of the cell; the protoplasm being reduced to a mere envelope and often invisible. There is no nucleolus, and the chromatin is distributed round the periphery in small, dense ovoid blocks: (Pl.7 fig.22a & b) apart from these blocks the nuclei are absolutely clear. These blocks are constant in number, and are heteromorphic: further, they are always in the resting nucleus arranged in pairs and the number of pairs agrees with that of the haploid number of chromosomes as counted in spermatocyte divisions, namely 13. Hence it is concluded that these blocks represent the condensed chromosomes. MALAQUIN describes these cells as having "nucléoplasme clair sur lequel se détachent 14-16 petites masses chromosomiques". No evidence is given for calling them chromosomal, and in his figures he does not demonstrate the regular ovoid shape which in reality distinguishes them so conspicuously from the chromatin masses in other nuclei: neither does he show them as existing in pairs.

When the neoblasts divide, their nuclei pass through a complicated prophase which is reminiscent of heterotypic gametocyte divisions. A series of figures is seen before the appearance of the spindle, in some of which there is a very intimate association between the members of homologous pairs. The stages seen in sections have been interpreted in the following order.

The condensed chromosomes of the resting phase elongate, but do not form a continuous spireme. They may unite together in groups, as there are generally about six threads visible (Pl. 7 fig.23). They pass later into a dense contraction figure, the various stages of which are shown in Pl. 7 fig. 24 & 25. At the beginning of contraction it is possible to see that a nucleolus is present, possessing the same staining properties as the chromosomes themselves. Also it is seen that the chromatin threads are more or less U-shaped, and orientated with their free ends towards one pole of the cell. The chromatin threads have a moniliform appearance during this phase, and sometimes there is an indication that the threads are double (Pl. 8 fig. ~~25~~ 25b & 26.): hence it is concluded that the homologous chromosomes are lying parallel to each other, and that the association between them is so close that it is difficult to distinguish the members of a pair. This phase then is exactly equivalent to a combination of the processes of synapsis and synesesis as they occur in gametocyte divisions.

While in this condition of contraction, the chromosomes condense again to a granular form, and on emerging enter another phase of distribution: they are still arranged in pairs, and the appearance now resembles that of diakinesis. The metaphase and final division of the cell now follow, during the whole of which the chromosomes are condensed in form. Pl. 8 fig. 28 shows the chromosomes collecting on to the spindle.

At some point during this division, a stage is interpolated in which the chromosomes are connected together by slender filaments - probably linin threads - in a linear series (Pl. 8 fig. 27). A thick moniliform figure results, curved to adapt itself to the space inside the nuclear membrane. Often a horseshoe curve is seen, but S-shapes, and other irregular curves are also found. Thirteen blocks are countable in this series: sometimes they are distinctly double, but usually no line of division can be found in them. When they appear single, it is assumed that each block represents a pair of chromosomes so closely approximated that - at least in the stained preparations - there is no visible demarcation between them. This phase illustrates the heteromorphism of the chromosomes particularly well.

The preceeding description applies to the neoblasts whether they are in the region of activity connected with bud-formation, or whether they are proliferating in the sexual somites and acting as oogonia or spermatogonia.

In previous cytological literature there are descriptions of chromosomal figures which agree in one or more particulars with those observed in the neoblasts of *Filograna*. The precise degree of comparison is variable, but in no case is there found a nuclear cycle in non-gametocyte divisions which corresponds in all stages with that just described.

The previous papers will be discussed in two groups for the sake of convenience, although these will overlap to some extent. Firstly the literature dealing with the persistence of chromosomes in resting nuclei (i.e. prochromosomes) is reviewed: secondly the records of association between homologous chromosomes, i.e. pairing of chromosomes, at times other than during reduction divisions, are compared with the present case. Only those particular papers which show close cytological resemblance to the neoblasts of *Filograna* are mentioned here: a fuller summary of all the work which has a theoretical bearing on both subjects is given by WILSON (1925).

Prochromosomes of more or less convincing appearance have been described in both somatic and germ cells: from these descriptions a series could be built showing at one end, homogeneous, clear or granular nuclei, and at the other, nuclei in which the whole of the chromatin is condensed into the chromosomes: intermediately would be the types in which the chromatin is more or less concentrated into distinct blocks, or at centres, and in which these are more or less constant in number. The diagrammatic black-and-white appearance of the neoblast nuclei in *Filograna* represents the final stage of this series.

The figures of STOUT (1912) and of TISCHLER (1910) show the closest approach to the present case. Both authors describe prochromosomes in somatic and germ cells, equal in number to the diploid chromosome number (see below for Tischler's remarks on fusion between members of a pair). ROSENBERG (1904 & 1909) finds chromatic bodies which he concludes to be prochromosomes, in resting nuclei of many plants. SYKES (1909) finds "fully formed chromosomes" in the resting somatic nuclei of many plants. OVERTON (1906 & 1909) states that prochromosomes are commonly found in the cells of the root tip in plants. The various cases that he gives show the variability in the degree of concentration on dispersion of the chromatin: in some of the nuclei, the chromosomes seem to be more of the nature of centres of concentration for the chromatin at the beginning of prophase, in others they are more definite and discrete. The descriptions of these authors are all confined to plant cells; cases of the existence of prochromosomes in animal cells are fewer. The chromosomes are described by KING (1902) as persisting in the ovarian egg of Bufo lentiginosus, and it is deduced from STEVENS (1907) that a similar condition is indicated. Although many kinds of cell are included among these cases, yet one gathers the general impression that prochromosomes are most commonly found in germ cells, cells in the tissues of the sexual organs, and embryonic cells (i.e. cells of young embryos and cells in the root tip).

The only other evidence of the persistence of chromosomes in animal cells, is derived from nuclei which show indications that an "architectural basis" of the chromosomes persist through interkinesis, though the actual visible - or stainable - structure, is changed in condition. Such evidence is obtained by comparing the position and arrangement of chromosomes at their first appearance in prophase, with that at the end of telophase: or alternatively by tracing chromosomal areas - or vesicular chromosomes - throughout the resting stages. Although these cases are theoretically similar, they are not discussed here, as they are not structurally identical.

Of the authors mentioned above, TISCHLER OVERTON (1906), and SYKES state that the prochromosomes are seen arranged in pairs. TISCHLER further emphasises the fact that the two members of some of the pairs are so intimately united that no dividing line can be seen between them. This explains why the number of prochromosomes counted sometimes is less than the diploid number, and the nuclei are called by TISCHER "pseudohaploid". There is little, if any, sign of this union in *Filograna* in the resting neoblasts, but as described above, it occurs at one stage of the prophase (Pl. 8 fig. 27). STEVENS (1910) describes a paired arrangement of the chromosomes in resting germ cells in Culex, the pairing occurring in telophase and lasting until the next prophase. METZ (1914) also HANCE (1917) mention the possibility that this might happen, STOMPS (1911) also thinks that the chromosomes may exist in a paired, or parallel, condition during interkinesis, as when they first come into view at the beginning of prophase they lie side by side.

There are authors who oppose the view that the darkly staining chromatic bodies which are seen in resting nuclei represent individual chromosomes. The paper of Digby (1914) may be mentioned among these, as it summarises very completely the opponent's point of view up to that date. In spite of what is said in the text of Digby's paper, the figures nevertheless do suggest prochromosomes in a marked degree, and even show the existence of the darkly stained masses in pairs. No definite opposition to the prochromosome theory since 1914 can be found.

The association in pairs of chromosomes during various stages of division is known from a considerable number of authors. SUTTON (1902) figured equatorial plates of spermatogonial divisions of *Brachystola magna* in which it was not only demonstrated that the chromosomal complex consisted of a double set of heteromorphic elements, but also that on the spindle they were arranged in pairs - the two of equal size lying closely adjacent to each other. MONTGOMERY (1906) found that homologous chromosomes lay parallel to each other in the spireme. Later STEVENS (1908) working also on Diptera, stated that pairing occurs in follicle cells, spermatogonia, and some embryonic cells; the same author in 1910 produced more evidence of the same nature, and suggested that it might possibly be the case that pairing of homologous chromosomes occurred in each mitosis of the animal. SYKES (1908) working on Funkia, describes both the early prophase threads and also those in telophase as being double, both in reduction, and in somatic divisions. JANSSENS et WILLEMS (1908) describe a paired arrangement in the kineses of somatic cells in Alytes obstetricans. GATES (1908) describes the origin of chromosomes in Oenothera rubrinervia by transverse fission of the spireme, and figures them as lying at first in pairs: he also suggests that the members of homologous pairs were adjacent on the spireme. MULLER (1909) states that the chromosomes are paired in the anaphases and prophases of somatic divisions in the root tip of Yucca. STOMPS (1911) states that the chromosomes of the vegetative nuclei of Spinacia oleracea are paired on the equatorial plates, and his suggestion that they are also paired during interkinesis was mentioned above. Dehorne (1911) emphasises the constant association of chromosomes in pairs in somatic nuclei, in Salamandre maculosa. METZ (1914) says that the pairs of chromosomes exhibit a close association at all times in Diptera, and in 1922 produced further evidence in support of this statement: he concludes that the paired association of homologous chromosomes in the diploid cells, somatic as well as germinal, is characteristic of Diptera. HANCE (1917) states that the chromosomes appear in the early prophases of somatic divisions in Culex pipiens in pairs, and WHITING (1917) supports this, adding that the homologous chromosomes are still approximated in metaphase, in the case of the spermatogonial divisions. The pairing of prochromosomes as described by TISCHLER, OVERTON and SYKES has been mentioned above.

The theoretical conclusions to be drawn from this evidence may be completely summarised in the words of STRASBURGER (1907): "Die in der Kernplatten der diploiden Kerne der Erbsenwurzel nachuweisende Anordnung der Chromosomen zu Paaren ist nicht nur eine Stutze der Individualität der Chromosomen sondern auch ihrer Verschiedenheit".

There is another feature of great interest connected with the paired arrangement of chromosomes. It was mentioned that in the neoblasts of Filograna, there is a synapsis stage, in the course of which there is apparently complete fusion of the two members of a pair, and also that there is a well-marked synesesis. Both of these phases are usually connected with maturation divisions, but this is not a unique case of such a phase occurring in somatic cells. STEVENS in 1908 described the paired chromosomes in the spermatogonial divisions of Diptera as being twisted together. TISCHLER (1910) states that even in resting nuclei, the prochromosomes may be so closely appreximated that the dividing line between the members of a pair cannot be seen. METZ (1914 and 1922) in a paper on the Diptera gives the most emphatic statement on this subject, saying that "Each pair of chromosomes goes through what amounts to a synapsis in every cell generation" and that the figures of division stages often resemble haploid nuclei. He also states that in early prophase the association between members of a pair may be so close that they resemble a single element.

In none of these previously mentioned cases is there any suggestion of synesesis occurring, so in that in particular, the neoblasts seem to be as yet unique.

There is a permanent controversy among cytologists concerning the synesesis stage of the spermatocyte prophase. By some authors it is regarded as an artefact, and by others as real. The present author considers that the appearance seen in sections is not an artefact. The chief reason for this conclusion lies in the fact that nuclei in the synesesis condition are seen adjacent to others in the earlier, more dispersed, spireme stages: if some of such nuclei were so altered during fixation as to have their chromosomes condensed into a contraction figure, it would be expected that neighbouring nuclei would be equally affected. Since the fixed nuclei in one small area show various stages of dispersion and contraction, it is considered that these represent actually different conditions.

The theoretical importance attached to the behaviour of the chromosomes in these unexpected ways is very great. The occurrence of synapsis entails the possibility of exchange of material - or of crossing over - in somatic cells, and the bearing of this on facts of heredity is obvious. Previous authors have emphasised the connection between the results of their observations upon problems connected with hybrids, and graftings, but their importance in interpreting normal questions of genetics is no less.

In conclusion, it is necessary to refer to two previous authors who figure a stage similar to that drawn in Pl. 8 fig. 27. GATES describes a comparable condensed, linear arrangement, and STOUT gives a second case, terming it a "discrete spireme", the term is defined as indicating that the individual chromosomes are clearly demarcated on the spireme. In STOUT'S case there is a pre-synaptic univalent spireme, and a post-synaptic diploid spireme - this second corresponding to the case of Filograna.

Although there is already an enormous mass of literature on the subject of chromosomes, yet it is considered that an addition to this is not superfluous. There is such great theoretical importance attached to the chromosomes from the genetical point of view, that it is very desirable that their behaviour during all phases of all the cell generations during an animals life, should be accurately known.

The greater part of previous chromosomes literature deals with germ cells, but from the foregoing paragraphs and from previous similar work, it is evident that there is reason to suppose that certain non-germinal cells may have a bearing upon some questions of heredity. There is obviously a possibility that interchange of chromosomal material (i.e. crossing over) may occur in the neoblasts of Filograna, hence it follows that the chromosomes of the mature germ cells of a bud (when it reaches a sexual phase), are not necessarily identical with those of the germ cells of the parent stock.

Apart from this special problem, the present case is a definite addition to the literature which supports the theory of individuality of chromosomes. This very fundamental question is still not settled beyond dispute. T.H. MORGAN in 1924 wrote "thus because cytologists have not yet been able to prove beyond all question the "individuality" of the chromosomes, the geneticist is left without the support that his evidence calls for". Similarly Mc CLUNG in another section of the same volume writes "There are those, however, who discount all the implication of the ordered events of the mitotic process, and deny the conception of the chromosome individuality because the physical state of the chromosome changes during the period of intussusception or growth between cell divisions ....It is not in the nature of the case that proofs of chromosome individuality supplied by unchanging form can be furnished ...."

These two quotations show the necessity for further investigation of the chromosomes and it is hoped that the present piece of work will add one more factor towards the final inteppretation of a few of the chromosome problems.

Differentiation of the nuclei produced by the proliferation of the neoblasts proceeds according to the ultimate fate of the cells produced. After division, the nuclei at first pass into the typical neoblast resting condition, and then, in the case of all except those cells which will remain as neoblasts, begin to differentiate. The first stages of differentiation, whether towards forming bud tissues, phagocytes, chloragogen or genital cells, are fundamentally similar. The direction in which differentiation proceeds seems to be determined by the position of the cell in the body, or, in the case of regenerating tissues, by the position which the cell ultimately occupies, after its final settling. The cells produced by the neoblasts are capable of producing any kind of tissue, independently of whether it is regarded as theoretically ectoderm, mesoderm, or endoderm. The functional equivalence of neoblasts and germ cells is further emphasised by the fact that sometimes an oocyte is seen in one of the bud somites amongst the histolysing mass.

The presence of small nucleoli in nuclei which still show signs of the pairing of the chromosomes, is taken as the first sign of differentiation of the neoblasts. Later the chromosomes lose their visible and discrete identity, and the nucleolus enlarges. Pl.5+7 Figure 20 & 21 show these stages of differentiation. Pl. 6 fig. 18 & 19 show the development of the coelomic sacs at the hind end of a bud.

The neoblasts are described by Malaquin as mesoblastic, but it is doubtful whether they can be said to belong strictly to any one of the three primary cell layers. Malaquin has traced them into the anal papillae, but it is possible to trace them even among the most terminal cells of the outer, posterior wall of the papilla. At the extreme hind end of the papilla is a gap in the thickness of the external cell layer, and here are found a few neoblasts with the typical resting (or sometimes active) nuclei (Pl. 6 fig. 18 & 19). The nerve cords extend through the papilla to this terminal point, and the neoblasts are found, as in the abdomen, adjacent to - and dorsal to - these nerve fibres. Also, between the last intestinal - ciliated cell, and the first ectodermal - non-ciliated - cell, are inserted more of these undifferentiated neoblasts. Although these have not certainly been seen in division, it is probable that they are concerned in providing the cells required for the elongation of the e~~ct~~ndoderm and of the ectoderm. The particular situation on the series of neoblasts where there is proliferation to produce the new coelomic sacs is marked * in Pl.6 fig.18 : it is immediately anterior to the solid mass of cells in the papilla.

The whole of the histological processes involved in the formation of a bud show that there is in this method of reproduction an entire disregard of "cell layers". Further, this description has made intelligible a previous remark concerning growing points. It was said that when a bud is developing, it does not in reality possess two growing points. Descriptions of bud formation generally convey the idea that the development of the anterior end of the bud is the result of the activation of a growing point removed from the hind end: this is not strictly accurate. The increased activity is shown by all the posterior segments equally and the formation of the head is not due to proliferation of segments quite in the ordinary sense. Cells accumulate here, but there are comparatively few cell divisions taking place: those that are seen are scattered throughout the tissues, and the cells dividing are by this time more or less differentiated. On the other hand, individuals which begin to regenerate the hind end while the bud is still attached, do have a second growing point, but this is terminal in regard to the future individual, and hence in no way abnormal. It would be interesting to know whether, in those ~~bases~~ genera which are able to regenerate a very large number of anterior segments, the neoblasts of the original segments are capable of producing them all. For example - ALLEN (1923) describes an individual <u>Procerastea</u> in which 31 segments were regenerated anteriorly from an original piece of three segments: it would require tremendous activity of the neoblasts to produce such a mass of new tissue.

It is interesting to compare the results of HYMAN (1916) and HYMAN AND GALIGHER (1921) on axial gradients in budding Oligochaetes, with the course of events in Filograna. According to HYMAN, the first sign of the production of a bud in Aeolosoma is the appearance of a zone of high metabolic activity in the neighbourhood of the region where the bud head will arise; later this increase in activity spreads over all the bud segments and becomes more strongly marked, so that at the time of freeing - or soon after - when dedifferentiation of tissues and reorganisation are taking place, the gradient all along the bud is higher than at any point in the stock. In the Naids however there is no early increase in metabolic rate at the plane of fission, but later when differentiation of tissues is taking place before and behind this level, the rise is detected: with the development of the bud head, the bud develops an independent gradient similar to that of a non-budding individual. This second case in which there is no initial rise at the plane of fission, agrees with what one would except to find in Filograna. HYMAN mentions that the gradient of Autolytus is quite like that of the Naids, but does not mention Filograna. The early anterior rise in the case of Aeolosoma may be due not so much to activity of the region of the bud head, but rather to the region immediately in front of this where there is active proliferation of neoblasts taking place (HÄMMERLING 1924, 1 & 2)

This piece of work has been performed partly in the Zoological Department of Bedford College, London, and partly at the Marine Biological Laboratory, Plymouth. I wish to thank Dr. E.J. Allen, F.R.S., Director of the Plymouth Laboratory for his constant assistance during the course of the work; also I am indebted to Professor J.H. Ashworth, F.R.S., Professor of Zoology in The University of Edinburgh for reading and criticising the manuscript.

I hope to follow this work by an investigation of the histology of the regeneration of polyps in Polyzoa, after the formation of "brown bodies". There is some biological similarity between the processes of bud-formation in <u>Filograna</u>, and the course of events associated with "brown bodies" in Polyzoa. A comparative histological study of the disintegration and regeneration of these polyps would be of great interest, particularly as the Polyzoa are not entirely unrelated to the Serpulids.

SUMMARY.

1. Data are given which show that in Filograna there is much variation in many of the external features, including those which are supposed, according to some authors, to distinguish Filograna from Salmacina. Hence it is concluded that these two forms are not separate genera but should be both included in the genus Filograna.

2. The course of the cil~~l~~iary currents in the tube is as follows:- water enters the posterior end of the tube; when it reaches the thorax, it is all directed dorsally by the thoracic membrane and passes out of the tube by the dorsal portion of the anterior aperture. The branchial (feeding) current collects from a more ventral field (Text figure 2)

3. The longitudinal muscles are well developed : their anterior sub-divisions form the muscles of the collar and branch~~a~~ie. Circular muscles are visible in the achaetous (i.e. post-thoracic) zone only. It is suggestedthat these act as a compression pump on the peri-enteric sinus, and drive the blood in this forward into the branchial filaments to expand these - or at least that they maintain the blood there under pressure. The gut musculature consists of a sheath of circular fibres surrounding all the pre-abdominal

region of the canal. In connection with the unicini there is an interesting musculature mechanism: a layer of turgid vacuolated cells in the parapodial wall is arranged to act as a passive, spring-like opposition to the retractor muscles of the uncini. (Text fig. 8).

4. The central nervous system is of a primitive Annelid type. There are two ventral cords widely separate, in close contact with the epidermis. Each Branchial filament is supplied with two nerves, one internal or mesial, and the other external or lateral: the latter bifurcates in each filament. (Pl.3 fig. 5).

5. The blood spaces are of the nature of open sinuses, except anteriorly, where true tubular vessels are present. The main, peri-enteric sinus surrounds the gut from the hind end of the proboscis to the anus and is a continuous uninterrupted cavity. The blood in it spreads into the mesenteries and dissepiments, giving rise to dorsal, ventral, and inter-segmental channels: these are open canals with no intrinsic epithelium. The blood in them spreads in an extremely thin film over the basement membrane of the epidermal cells. Anteriorly there is an annular vessel round the proboscis, from which branchial, and collar, vessels arise. (Figures 1,16,15 & Text fig 9). The walls of the vessels and sinuses are formed by the membranous

peritoneum only. Movement of the blood is effected by anti-peristaltic contractions of the thoracic gut muscles and by movements of the body: the circular muscles in the body wall probably aid also.

The form of the blood system is considered to support the hypothesis that the cavity of the blood system in Annelids represents - or has been derived from - the blastocoel of the embryo.

6. One pair of excretory organs only is present, lying in the collar somite, and having the form of simple S.shaped tubes: the mesial ends of these unite ~~from~~ to form a common terminal duct, which passes dorsally through the prostomium and opens terminally. Internally there is a granular enlargement of the tubes to form the "glandular sac"; a pore places the cavity of the "sac" in communication with the coelom of the somite anterior of the collar, and hence is a nephrostome. A large flame (200µ in length) arises in the sac near the nephrostome and beats into the anterior coelom: the cillia in the excretory duct are also long and flame-like. (Figures 1 + 12, + Text fig 10).

7. Buds are abundant during spring and early summer: sexual individuals are mature (☿) and buds rarer during summer and autumn. A stock bearing a bud is generally asexual, and the bud itself is always asexual. The eggs develop in the calcareous tubes of the parent until the embryo is almost ready to settle. Embryos devloped during early summer enter a sexual phase in the same season, those produced later reach their first reproductive phase (asexual) next spring. Individuals last more than one year, hence after a sexualphase, there is a return to asexual reproduction in the next spring.

8. In the formation of a bud the anterior region, including branchiae, prostomium, collar and the next succeeding somite, is formed by an outgrowth of new tissues at the plane of fission. The original stock somites posterior to this are transformed into bud somites, theanterior six or seven being changed from abdominal into thoracic segments. (Pl. 2 fig.2)

9. The interior of a bud is filled with opaque, segmental blocks, composed of active phagocytes and embryonic cells. These persist for some time after the bud is freed, gradually diminishing in size and density. (Pl. 2 fig.2 &
(Pl. 1 fig.3).

13. The production of a bud entails a complete histological disintegration and regeneration of bud tissues, and is accompanied by a similar but much less complete process of rejuvenation of stock tissues. (Pl. 5 + 6 fig. 15 & 16 and microphotographs). The cells concerned in the phagocytosis and replacement of tissues are derived from neoblasts in the ventral body wall. The cells produced by these neoblasts give rise to ectodermal, endodermal, and mesodermal structures indifferently, and are probably identical with the cells which are responsible for the growth in length of all the tissues of the animal at the posterior growing point, independently of budding phenomena. (Pl. 6 fig. 18 & 19).

Neoblasts are identical with the sex cells (i.e. oögonia and spermatogonia).

Nuclei of neoblasts in the resting stages possess definite chromosomes - prochromosomes - arranged in pairs in an otherwise perfectly clear vesicle. (Pl. 7 fig:22).

In every division, the neoblasts pass through a contraction phase which strongly resembles the "synesesis" phase seen in gametogenesis, and is either preceded by, or followed by, a period of synapsis during which the individuals of a pair are so intimately associated that no ~~H~~ line of division can be seen between them. (Pl. 7 + 8 fig. 23 - 28).

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EXPLANATION OF LETTERING OF PLATES.

A	anterior.
al.c.	alimentary canal
an.	anus
a.p.n.	nuclei of cells of the interior of anal papilla.
ax.v.	blood vessel in branchial axis.
b.	brain
bl.s.	blood sinus.
br.c.	cell of branchial axis.
br.fil.	branchial filament
br.g.	branchial nerve ganglion.
br.v.	branchial vessel.
c.c.	coelomic cavity.
c.c.c.	coelomic cavity of collar somite.
c.c.P.	most posterior coelomic cavity.
c.ch.	collar chaetae
ch.	chaetae
ch.c.	chloragogen cells
cil.b.1.	first transverse ciliated band
cil.b.2.	second ditto
cil.d.	ciliated duct of glandular sac
cil.g.	ciliated groove on branchial filament
col.v.	blood vessel of collar
con.	peri-oesophageal connectives.
com.	nerve commissure.

D.A.	dorsal angle of thoracic membrane
ec.n.	nucleus of ectodermal cell
en.n.	nucleus of endodermal cell
f.gl.	fat globules
gl.s.	glandular sac
gl.s.ap.	cells at the aperture of excretory organ
gl.sh.	gland shield
G.P.st.	growing point on the stock
gr.	granules in phagocytes of bud
lat.cil.	large lateral cilia on branchial pinnules.

mes.	mesodermal masses of histolysing tissue in bud
mes.st.	similar masses in the stock
mit.	mitotic divisions of gut cells
mth.	mouth.
M.br.ext.I & II.	external branchial muscles.
M.br.int.	internal branchial muscles
M.degen.	disintegrating muscles.
M.d.c.	depressor muscle of collar
M.d-v.	dorso-ventral fibres.
M.d-v.a.	transverse fibres between proboscis and branchial base.
M.e.a.	muscle of angle of collar.
M.e.c. 1,2 & 3.	erector muscles of collar.
M.e.ch.p.	posterior erector of collar.
M.g.	circular fibres round alimentary canal
M.l.d.	dorsal longitudinal muscle.
M.l.v.	ventral longitudinal muscle.
M.l,m.	mesial ventral longitudinal muscle.
M.pr.	anterior termination of M.l.m.
M.r.ch.	retractor~~s~~ muscles of long chaetae.
M.r.ch.p.	Posterior accessory retractor of chaetae
M.s. 1,2, & 3.	anterior septal muscles.
M.tr.a.	anterior cephalic transverse muscle fibre.
M.unc.	uncinal muscle.
M.unc.p.	accessory uncinal muscle.

nb. e-e.	neoblast nuclei at junction of ectoderm and endoderm.
nb.t.	terminal neoblast nuclei in anal papillae.
n.br.c.	nuclei of branchial axis cells.
n.div.	nephrostomical diverticulum.
n.fl.	nephrostomial flame.
N.br.ext.	external branchial nerve.
N.br.int.	internal branchial nerve.
N.c.a.	anterior collar nerve.
N.c.p.	posterior collar nerve.
O.	oöcyte.
P.	indicates posterior.
p.ach.	parapodia on achaetous zone.
p.b.v.	blood-vessel in pinnule.
per.	peritoneal cells.
p.g.	posterior cerebral ganglion.
p.i.s.	peri-enteric sinus.
pr.	prostomium.
prb.	proboscis.
prb.b.	proboscis of bud
prb.gl.	proboscis gland
pre-c.c.c.	coelomic cavity of pre-collar somite.
post-c.c.c.	coelomic cavity of post-collar somite.

r.v.	ring vessel
s.	dissepiment
s.n.	most posterior septum in stock
s.~~n.1~~ n+1	next septum posterior to this in original series
s.p.	most posterior septum in the individual
s.oe.g.	sub-oesophageal ganglion
th.m.	thoracic membrane
unc.	uncinus
v.mes.s.	ventral mesenteric sinus
v.n.c	ventral nerve cord.

DESCRIPTION OF PLATES.

Fig.1. Semi-diagrammatic view of anterior end seen from the dorsal surface.

Fig.2. External features of the successive stages in the production of a bud. Figures drawn from living animals with the aid of a Leitz Zeichen-Okular.

A. Dorsal view of hind end of stock bearing a very young bud.

B. Bud of the same age as A., drawn as seen when focussed mid-way between the dorsal and ventral surfaces, to show the coelomic cavities.

C. Ventral view of slightly older bud, at the stage of development at which it possesses 4 pairs of branchial filaments.

D. Slightly older bud with the branchial filaments pinnate and the anterior bud somites complete. Both dorsal and ventral features are shown at the region of junction between bud and stock.

E. Lateral view of bud and kind end of the stock, of a bud of the same age as D., to show the attachment of bud.

F. Ventral view of a bud attached to a stock which has commenced to regenerate segments posteriorly, while the bud is still developing.

G. Dorsal view of the same individual.

H. Dorsal view of a stock bearing a bud of the same age as C. showing fat globules in the coelom of the stock.

K. A stock with a bud attached, showing an extreme case of degeneration of the stock tissues.

Fig. 3. An individual which has been recently freed as a bud, to show the features characteristic of this phase of the life history: drawn from a living animal.

Fig. 4. A stock which has recently produced a bud and is now regenerating its hind end: dorsal view - from a living animal. The arrow indicates the plane at which the bud was separated.

Fig. 5.
a. T.S. of a branchial filament.

b. T.S. of a branchial pinnule.

ig. 6. Drawings obtained by superimposing consecutive serial sections, to show the musculature of the anterior region.
a. is lateral to b.

ig.7. Drawing of the anterior end obtained by superimposing several consecutive horizontal serial sections, to show the musculature, excretory organ etc. : the right half is more dorsal (i.e. superficial) than the left half.

ig.8. Three thoracic parapodia seen in horizontal section. Several consecutive sections are superimposed, and the anterior parapodium is shown at a more dorsal plane than the posterior one.

ig.9. Median longitudinal section of prostomium and proboscis

ig.10. Horizontal section of a blood sinus on an abdminal dissepiment to show the enlargement of the blood space, and chloragogen cells.

ig.11. Horizontal section (several consecutive sections combined) through prostomial and collar region, at a plane ventral to that figured in Fig.7.

ig.12. Diagrammatic representation of the anterior end in horizontal view, to show relationships between glandular sac and coelomic cavities, also nervous system and alimentary canal.

Fig.13 Diagrammatic lateral view of prostomium and proboscis to show the brain and cerebral (i.e. branchial) nerves.

Fig.14. Longitudinal section (not median) of prostomium and proboscis, to show relationships between nervous system, blood vessels and musculature.

Fig.15. Horizontal section of hind end of a stock with abud, showing disintegration of muscles in the stock as well as in the bud. The posterior end of the stock has commenced to regenerate new segments while the bud is still attached.

Fig.16. Horizontal section through the region of attachment of bud to stock; age of bud approximately as in Fig. D. Portion enclosed between dotted lines is enlarged further in the next figure.

Fig.17. A further enlargement of a portion of Fig. 16 q.v. Drawn with the aid of a camera lucida, using a 1/16" oil immersion lens. Flemming-without-acetic preparation.

Fig.18. Horizontal section through the anal papilla and posterior coelomic cavity of a bud to show the terminal neoblasts. Cell nuclei only are figured, the cell outlines being omitted. See also Fig.19.

Fig.19. As Fig.18, drawn from an individual showing particularly neoblasts at the line of junction of ectoderm and endoderm: terminal neoblasts also seen. Figg.18 and 19 both show early stages of differentiation of neoblasts into tissue cells, before the nucleolus appears.

Figure 20 (on Pl. 5) Horizontal section through septal sinus of bud, showing neoblasts migrating, and differentiating.

Figure 21. Neoblast nucleus at early stage of differentiating into endodermal nucleus; a nucleolus is present and the chromosomes are losing their paired arrangement.

Figure 22-28. Stages of prophase of division of neoblast nuclei. The small, outline drawings represent camera-lucida tracings made under a 1/16" inch oil immersion lens, the scale being indicated. The enlargements were made without aid from the camera lucida.

Figure 22a & b. Resting neoblast nuclei, showing the condensed form and paired arrangement of the prochromosomes.

Figure 23. Spireme stage.

igure 24. Beginning of contraction - or "synesesis" phase.

igure 25 a & b. Slightly later phase, showing "synesesis" complete.

igure 26a. Portion of a nucleus at the stage of transition from "synesesis" to "diakinesis".

26b. Portion of thread from the other half of nucleus, showing its double nature.

igure 27a & b. Stage of "discrete spireme" in which the chromosomes, in pairs, and more or less fused, are arranged in a linear series.

igure 28. Early metaphase.

Fig.29-33 Micro-photographs. In all cases, fixation was with Allen' chromic-urea-Bouin, and subsequent staining, with iron haematoxylin.

Fig.29 L.S. of individual showing first signs of budding: the thickened patch of epidermis is the beginning of the bud head, and the collection of phagocytes is seen internally. The stock has sexual organs in this case - three segments testis, and a few small ova posterior to this.
Photographed under $\frac{2}{3}$" obj:

Fig.30 H.S. of hind end of stock with bud showing an extreme case of degeneration of bud tissues. ($\frac{2}{3}$" obj)

Fig.31 Oblique section of hind end of stock and bud head, showing disintegration of the stock tissues (alimentary canal and muscular tissues). ($\frac{1}{3}$" obj)

Fig.32 H.S. of hind end of stock showing histolysis of tissues, also, the presence of large ova-like cells in the coelom. The new posterior growing-point of the stock is seen on th right behind the last parapodium. ($\frac{1}{4}$" obj)

Fig.33 H.S. of a bud after freeing, showing histolysis and regeneration of tissues (alimentary canal and muscles) still proceeding. ($\frac{1}{4}$" obj).

THE EARLY PROPHASES OF THE OÖCYTE AS SEEN IN LIFE IN *Obelia geniculata*.

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account has been made: there are previous descriptions of the observation of chromosomes in other types of living cells which are referred to later in the historical section of the paper.

All the figures (with the exception of the spermatocyte nucleus drawn in fig. 23) were drawn from life, with the aid of a Leitz Zeichen-Okular, under a 1/6 inch objective. In most cases the medusae were examined within the first few hours after they were brought into the laboratory, but occasionally they were left in an aquarium during the night and examined the following morning.

During the sexual season, each gonad contains a group of oöcytes at different stages of growth: small resting cells are present always, and there are generally about six larger oöcytes in which the nucleus has passed from the resting condition into the active pre-maturation phases. Figure 1 shows a typical ovary drawn from life semdiagrammatically, as seen when focussed deeply below the surface. The oöcytes are arranged in a single series around the exterior of an endodermal pouch: there is one such pouch developed on each of the four radial canals; and the four lobes thus formed project from the subumbrellar surface and hang freely into the concavity of the bell. Externally the oöcytes are covered by a thin ectodermal cellular layer, hence they occupy a position between endo- and ecto-derm. The youngest oöcytes are situated proximally at the base of the gonad, and the oldest are the most distal: frequently instead of the symmetrical arrangement seen in the figure, there is one large terminal oöcyte.

In the young resting oöcytes at the base of the ovary, * the nucleus is almost entirely filled by the nucleolus (figs.(1 and 3). This nucleolus is refringent in appearance, and though it is almost colourless, appears to have a slight greenish-grey tint against the perfectly colourless surroundings. It is vacuolated to a variable extent: there may be one large vacuole or many small ones, and the pattern of these changes under observation (figs. 2 - 5).

As the cell increases in size, the nucleus enlarges also, but the size of the nucleolus remains practically unchanged, its diameter being approximately 15μ. When the cell reaches a diameter of 50 - 75μ (nucleus being c.30μ) differentiation of the nucleus commences and a series of typical oöcyte prophases follow. The chromosomes become defined as such, and later disappear again, leaving the nucleus clear.

The first stage in the differentiation of the nucleus is the elongation of the circular nucleolus: the details of this process vary in different cases, but the final result is the transformation of the nucleolus into an elongated ribbon, which by virtue of its subsequent behaviour, must be considered to be a spireme. Figures 6, 7 and 8 show three oöcytes in which the nucleolus is in the process of elongation. Figure 6 shows the type most frequently seen, in which at the very first stage, the nucleolus assumes a C-shape: Fig. 8 shows a slight modification of this type of nucleolus. Fig. 7 shows a cell whose nucleus is at approximately the same age, but in which the necleolus is producing a long slender ribbon instead of a short thick one. This kind of ribbon seems to be derived from the nucleolus by a kind of spreading process which suggests the exact reverse of what would happen if a coiled length of some plasticene-like ribbon were taken in the hands and compressed into a smooth ball. The ribbons derived from nucleoli such as are figured in Figs 6 and 8 elongate more or less subsequently, becoming correspondingly slender at the same time.

During the next phase, the spireme breaks up by a series of transverse fissions, and finally in place of the continuous ribbon there are spherical fragments of various sizes scattered through the nucleus. The details of the process of fragmentation very considerably. Figures 9 to 13 illustrate nuclei in which the breaking up of the spireme is in process. In fig 9 the nucleolus is still elongated and is seg~~ma~~menting regularly at one end; such a nucleus might be a later stage of a nucleus such as that in fig. 6 or in fig. 8. Fig. 12 shows an almost unique case in which the nucleolus began to fragment without undergoing any initial elongation. Fig 10 shows a later stage of the Fig. 7 type of spireme: the coiled knot-like formation is now less compressed, and the coil has segmented into sections- the divisions occurring simulataneously throught the entire length of the ribbon.

Figure 13 differs from the previous four in that the spireme has become more elongated, also in the fact that the divisions are not all happening synchronously, hence several long pieces of spireme are co-existent with numbers of small fragments: such irregular nuclei are very common.

The completion of the fragmentation phase leaves the nuclei in the condition seen in figures 11, 14, 15, 16, and 17, The spireme has entirely broken up into more or less circular fragments, and it is concluded that each fragment is a pair of homologous chromosomes. The evidence for calling them chromosomal lies in the fact that when ideal cases of the phase are found (i.e. cases in which the divisions synchronose accurately), the number of these fragments is constant: further the fragments are not of equal size but show a constant pattern of heteromorphism.

It is assumed that these two features are actually characteristic of chromosomes.

In figs. 14a to 17a, the chromosomes are sketched in the nuclei to show their arrangement, but are actually rather smaller than they should be. They were drawn so to add clearness to the drawings; in figs. 14b to 17b, the same chromosomes are drawn with the aid of a camera lucida and arranged in graduated series for comparison. There is in each case one element larger than the rest, and there are two almost minute fragments; between these two extremes there is a fairly even gradation. Two or three of the fragments are always conspicuously larger than the rest. The number of chromosomes counted at this phase is 17, and as the elements are later shown to be bivalent, this is equivalent to the haploid number.

Figure 11a is a rather special case of a nucleus at this particular phase. It is unusual to find such an early breaking of the spireme as this; the divisions must have occurred at the commencements of unravelling of a spireme of a type intermediate in form between those of figs. 6 and 7. In fig. 11 indication of 15 blocks could be found, hence it is an almost typical example of a completely segmented spireme.

In the following stages of growth each of these bilvalent chromosomes divides into two equal halves. At first the two halves resulting from the division remain adjacent, but later they separate, so that finally the fragments are evenly distributed over the nucleus. Figures 18 to 22 show such nuclei: various degrees of pairing and of dispersion are seen, and as is expected, heteromorphism parallel to that of the previous phase is conspicuous. Figs. 18b and 19b are camera lucida tracings of the chromosomes of the corresponding nuclei. Fig 18 has 34 chromosomes countable, but only the larger ones were accurately traced. In fig. 19, only 30 chromosomes are seen and these are all drawn. In fig 20 the larger fragments were also traced: in this case there are 31 small, presumably univalent elements, and two larger ones which are probably still bivalent but showing signs of fission. Hence the number is estimated at 35 here. In figs 21 and 22, there are 34 chromosomes present in each.

It is therefore concluded that 17 and 34 are the n and 2n (i.e. haploid and diploid) numbers respectively for the chromosomes of Obelia geniculata.

The most interesting point in connection with the division of the bivalent chromosomes is the indication that the large chromosome seen in the bivalent group represents an unequal (XY) pair. In the univalent condition the largest chromosome is often irregular in form, most commonly hammer-shaped or wedge shaped; it generally lies in the centre of the nucleus. In the nucleus represented in figure 21, it is believed that the large chromosome had divided immediately before inspection and that the two chromosomes in the centre of the field, one larger and triangular and the other smaller and spherical resulted from the division: these two chromosomes were drawn as accurately as possible, with the camera lucida, but the rest of the elements were sketched free-hand. It should be emphasised here that the paired arrangement of the individual chromosomes for some time after their separation is much more conspicuous and convincing in real life than it is in the figures: this is necessarily so, for the chromosomes are in reality distributed through the interior of a spherical chamber, and hence are separated from each other by distances of depth as well as the lateral distances which alone can be represented on a plane surface. The appearance of the nucleus from which fig. 21 was drawn was such that it left no doubt that the two chromosomes under discussion were really members of a pair, although no further proof can be produced. Probably the commashaped form in fig. 20 represents the corresponding XY pair in this nucleus, since, as mentioned above, there is indication that this is a bivalent element.

After the chromsomes have become defined in this manner, they disappear from view again and leave the nuclear vesicle quite clear. The disappearance is due to the further fragmentation - or disintegration - of each chromosome into a group of small globules. The fragmentation may occur gradually, in which case the fragments become steadily smaller and at the same time more numerous: on the other hand each chromosomes may resolve itself at one moment into a group of minute globules which at first remain as a group, but later become dispersed. As a result of this process the chromosomes lose their discrete visible individuality entirely. WILSON (The Cell 1927) discusses the apparent fading of the chromosomes at this stage of oögenesis and mentions the fact that several authors describe this particular kind of temporary dispersion of the chromosomes. He expresses the opinion that the chromosomes "do not disappear by breaking up into a structureless magma or mass of fine granules, as some observers have concluded; there is reason to believe that this account rests on faulty technique." In the present case there can be no question of imperfect technique, and I feel convinced from personal observation of these nuclei that the condition is normal and not pathological.

The series of stages described and figured here are not always achieved in the orderly and ideal sequence in which they have been described. The phases often overlap each other to a considerable extent, so that nuclei are constantly found in which elongated sections of spireme are present together with numbers of bivalent, or even univalent, chromosomes. Further the stage characterised by the presence of the bivalent elements may be masked by the precocious division of a few of these elements, so that a nucleus such as that drawn in fig. 16 results: in this there are four fragments in excess of the normal 17; these may have been derived from division of the smaller bivalents; sometimes they seem to arise by separation from one of the larger chromosomes. Then similarly the typical "chromosomal" phase in which the 34 chromsomes are displayed, may be omitted as an actual fact, and counting rendered impossible, owing to the early and irregular fragmentation of some individuals. However a sufficient number of typical stages has been found to demonstrate repeatedly the actual course of events.

Whether the final division of the bivalent chromsomes represents a further transverse, or a longitudinal division is not known. No trace of a longitudinal fission was ever seen in the spirime or in any portion of it; in view of the fact that irregularities are met with so frequently in all stages of the cycle of events, and that there is certainly an overlapping of the univalent condition of some pairs of individuals and the spireme condition of others, it might be expected that if the final division were longitudinal, some trace of the split might have been indicated occasionally as an anachronism. As this never occurred, it is concluded that the chromosomes are probably arranged in a single linear series on the spirime, members of homologous pairs being adjacent.

As mentioned above, this is an account of observations made on the living egg entirely, However after the whole story had been completed a number of medusae were fixed and mounted, either as whole mounts (Stained and unstained) or as sections. In the case of sections, fixation was with Flemming-without-acetic, Bouin, and Allen's chromic-urea-Bouin; subsequent staining was with either iron haematoxylin, iron haematoxylin and eosin, or safranin and lichtgrün. These preparations have confirmed every step of the above description, but, as regards nuclear characters, have added nothing. Then also, some male as well as female individuals were sectioned in order to attempt to count the chromosomes in the spermatotycte. It has not been possible to do this with certainty, but it is evident that the number of chromosomes countable in the spermatocyte divisions is in the neighbourhood of 15 pairs. Figure

23 was drawn from a spermatocyte nucleus in which it was possible to make an approximate estimate of the chromosomes. This helps to confirm the conclusion reached previously that the nucleolar fragments really do represent chromosomes.

There is one other small point worthy of notice concerning these oöcyte nuclei. On several occasions long needle shaped crystals were seen in the nuclei, though the nuclei appeared in every way perfectly healthy and otherwise normal. The crystals were long and slender, being equal in length to the diameter of the nucleus. They were colourless, and varied in number from on single crystal to a star shaped group~~s~~ of about two dozen.

HISTORICAL.

The nuclei of the oocytes of <u>Obelia</u> have been mentioned and figured by several previous authors. The earliest account is that of MEREJKOVSKY ~~(1885)~~ (1880 and 1883) who apparently observed them in life, and described the various phases in the divisions of the nucleolus: however he missed the fact that the fragments had a recognisable individuality in all the different nuclei. He mentions that at one stage in the processes of fragmentation there are "plusieurs dizianes" fragments countable in the nucleus, and that these divide further, producing finally about a hundred. The "plusieurs dizianes" no doubt refers to the 34 chromosomal elements: the author obviously regards this particular phase as noteworthy and of the nature of a landmark, and must have realised that the divisions were not entirely indiscriminate and steadily continuous.

TRINCI (1906) mentions <u>Obelia</u> in a paper on Coelenterate oöcytes, also <u>Phialidium</u>, which is described as extremely similar. He discusses other cases of fragmentation of nucleoli in Coelenterates and gives a bibliography of the subject. His observations on <u>Obelia</u> were made on serial sections, and he simply states that the nucleolus fragments, without discussing the significance of this. He considers that the crhomosomes exist in an unstainable condition during the growth stages. It is of great interest to notice that in describing these nuclei, TRINCI mentions that the number of fragments varies according to the size of the egg, but apparently he realises, as MEREJKOVSKY does, that the divisions and subdivisions do not follow each other in a perfectly continuous series, for he states that by the division of the nucleolus there result "più decine di corpi rotundi" These, (presumably the bivalent chromosomes) divide further and give more than a hundred fragments, then are entirely dispersed. In another place he states that "nelle ouva più grandi se ne incontrano talora anche una trentina" Hence he also recognises the occurrence of a 30, or "chromosomal" phase, but does not attach any importance to it.

JORGENSEN more recently (1913) has described the whole nuclear history again, and he gives a complete series of figures. His observations also were confined to sections, and he has in fact, added nothing to TRINCI'S previous account. He claims to see faintly staining, oxyphilic chromsomes in the larger nuclei, but although I have used the precise method described by JERGENSEN as showing the chromosomes (namely safranin and lichtgrün), I have failed to find them. This paper of JORGENSEN is quoted by WILSON (The Cell p. 354) as constituting strong evidence in favour of the view that crhomosomes and nucleoli are morphologically completely independent, whereas according to the interpretation of the present author Obelia is considered to provide the most striking demonstration of a case in which the two are not only dependent, but even identical.

Although this particular relationship between nucleoli and chromosomes is unusual, the present case is not unique. The nature of nucleoli and their inter-relationships with other cell structures have been discussed at great length by many previous authors, and most of these agree that "nucleoli" are not in all cases identical. Those of oöcytes are admittedly often different from those of somatic cells, but on ultimate analysis the difference seems to be one of degree rather than of kind. The conclusions of previous authors, in so far as they concern the present case, will be briefly reviewed.

FLEMMING in 1882 stated that the nucleolus and the chromatin network of the cell both have essentially the same function and that the chromatic figure formed at the commencement of division derives its substance from both the network and the nucleolus.

R. HERTWIG (1884, 1896 and 1898) established and emphasised the fact that two distinct components ~~parts~~ are visible in the nucleolus, chromatin and plastin. O.HERTWIG (1909) supports this view, and addsthat the strongly chromatic nucleoli are seen in "viele niedrigen Organismen." Such chromatic nucleoli contribute towards the formation of chrosomes in the prophases of cell divisions.

CARNOY also throughout all his papers acknowledges the existence of a type of nucleolus rich in chromatin (Nucléoles-nucléiniennes"), which furnish material for the formation of chromosomes in prophases. In his monograph of 1884 he particularly mentions oöcyte nuclei and says that their so-called nucleoli represent the nuclein-containing substance of the nucleus.

MACALLUM (1895) finds, in accordance with the previously mentioned authors that nucleoli contain chromatin, and moreover that in some cases they contain almost all the chromatin of the nucleus, and that when the chromatic filament forms, it does so at the expense of the nucleolus (or nucleoli). His evidence is particularly interesting as it is not based upon the notoriously capricious staining reactions of the ~~of the~~ structures in question but on more specific methods - namely chemical tests for the detection of iron.

WENT (1897) describes the transference of the nucleolar material to the chromatic ribbon in Leucogium aestivum.

In 1898 MONTGOMERY produced a large paper summarising the known facts and theories on the subject of nucleoli. He gives a very long bibliography, but his personal results have not much bearing on the present case. He states that he can find no "evidence in any cell that the nucleoli stand in any genetic relation to the chromatic elements of the nucleus," but previously he establishes the point of view that nucleoli which react like chromatin or which are formed from chromatin are not true nucleoli, and his remarks apply therefore to plasmatic nucleoli - or plasmosomes only.

JANSSENS & WILLEMS (1908) suggest a classification of the various types of nucleoli, and propose the term "chromoplastes" for those which originate from the chromosomes.

LUNDEGARDH (1912) considers that the disappearance of the nucleoli in dividing cells as the chromatic filament comes into view, is evidence that there is a transference of material from one to the other: he further adds that there is sometimes a morphological connection between the two. He also mentions, without further explanation that "bei vielen niederen Organismen die Crhomosomen aus Nukleolus-ähnlichen Körpern entwickelt werden."

The most recent paper on the general nature of nucleoli is that of TAMURA (1923) who concludes that nucleoli and chromosomes are of essentially the same nature. He states that nucleoli, chromosomes and the nuclear network are all composed of the same two substances one internal and oxychromatic, the other peripheral and basicchromatic. The ~~emsh~~meshes of the network according to TAMURA form intercommunicating channels through which the internal basi~~c~~chromatic substance can flow. According to this view, the nucleoli arise as a result of the accumulation of a large proportion of the internal substance at one point on the network, and the author considers that the presence of a basichromatic outline to the nucleoli is the rule. TAMURA adds that the substance of the nucleoli is gradually transferred to the chromosomes, in nuclear divisions.

All these authors agree that chromatin is present in nucleoli: there are others who describe a still more intimate relationship between necleoli and chromosomes, resembling more closy that which exists in the oöcyes of Obelia.

HARTMANN (1902) and GUNTHER (1904) describe the origin of chromosomes from the nucleolus. JORDAN (1910) describes a peculiar case in which the chromosomes appear in the nucleus, then subsequently fuse into a chromatic nucleolar mass, from which they later emerge again.

In the following cases, the relationships of the nucleolus are almost exactly the same as in the oöcytes of Obelia. BLOCHMANN in 1882 described and figured convincingly the divisions of the egg of Neritina fluviatilis, andhe states that the nuceolus "in mehrere Bruchstücke zerfallt."

further, he adds "dass die Elemente der Kernplatte aus Teilstücken des Nucleolus entstehen, Kann bie unserem Object keinem Zweiful unterleigen."

Spirogyra nuclear divisions have been described by several authors, most completely by MOLL (1893), who indicates that the chromatin filament is drawn out of the nucleolus, no trace of this remaining eventually.

R. HERTWIG (1894 and 1898) described the nuclear divisions of <u>Actinospherium,</u> and showed that the nucleolus becomes broken up at its surface into small groups of globules, each group being the beginning of a chromosome.

WILSON (1901) mentions a type of Echinoderm egg in which the chromosomes arise from the nucleoli, exactly, he says, as in <u>Actinosphaerium.</u>

GOLDSCHMIDT (1902) describes a series of events in <u>Polystomun</u> precisely the same as those occurring in <u>Obelia</u>.

KATHERINER (1904) finds a case agreeing with GOLDSCHMIDT'S in <u>Gyrodactylus.</u>

BROWNE (1913) describes similarly the dissolution of the nucleolus unto chromosomes in <u>Notonecta</u>, but in this case a plasmatic fragment remains at the end.

The conclusion reached after considering the accumulation of literature on the subject of nucleoli, is that there are almost as many types of nucleoli as there are nucleoli and that therefore it is impossible to establish a nomenclature to distinguish between them. It is considered most practical to retain the general term "nucleolus" and to define its precise meaning in every case.

The reason that TRINCI and JØRGENSSEN~~S~~ missed the essential chromosomal nature of the nucleolar fragments, was probably that they examined only sectioned gonads. The most obvious indication of the chromosomal character lies in the facts that the fragments are constant in number, constantly heteromorphic, and at one stage, paired. All these characteristic features are masked in section, because the nucleoli~~e~~ are so large in fully developed oöcytes that they extend through about ten sections of normal thickness (e.g. 5 μ :) hence the possibility of counting or of measuring them is lost.

The particular kind of short, thick spireme that is seen so often in Obelia is perhaps less common than the elongated coiled type. A somewhat similar type is described by STOUT in Carex, but this differs in having a monilform appearance. GATES (1908) described a still more similar case in Oenothera, in which the fragmentation of the spireme into pairs of chromosomes shows a strong resemblance to the presents case. GATES concludes that the two members of a pair were adjacent on the spireme, as also they are concluded to be in Obelia.

The last point of interest arising from these observations concerns the visibility of the chromosomes in life. Nucleoli are frequently visible, but chromosomes only rarely. In the oöcytes of <u>Obelia</u> both are identical in appearance and are equally distinct: they have the same greyish tint and show the same vacuolated consistency.

SCHNEIDER in 1873 described mitotic divisions in Platyhelminth eggs, and made his observations on living eggs which had been previously treated with dilute acetic acid: by this means he saw the chromosome filaments and observed their behaviour in metaphase and anaphase. He says that the filaments are not visible unless the eggs are treated with acid however.

HERTWIG (1898) figures dividing <u>Actinosphaerium</u> as seen in life, and although the actual chromatic filaments are not visible in the drawings, yet the general arrangement of the groups of chromosomes is clearly indicated.

The previous account which most nearly resembles the case of <u>Obelia</u> is that by MULSOW (1911 & 1912) of the spermatogenesis of <u>Ancyracanthus</u>. The author confirms all his observations on living material and he figures ripe sperms in which the chromosomes are plainly visible as small refractive spherical granules, very reminiscent of those of <u>Obelia</u>.

TISCHLER working on banana pollen (1910) made observations on both living and fresh material, and he particularly mentions that he saw the synapsis phase in the living cells.

Crhomosomes in living cells have also been described by CHAMBERS, and have been manipulated by him with the micro-dissecting needle. In some cases the chromosomes are said to come into view when the cell is injured with the needle but in others (CHAMBERS, SANDS 1923) are visible in the normal untouched cell. Also they have been seen in dividing cells in tissue cultures, as described and figured by STRANGEWAYS (1924).

It is considered rather probable, from reading the previous literature, that the opportunity for observing chromosomes in living cells may be greater than is realised - particularly among the so-called lower groups. Observations of this kind form the only satisfactory means of settling controversial points on chromosomal questions, particularly those which relate to the formation of artefacts, and to the sequence of events in complicated nuclear changes.

The very large size of the chromsomes of *Obelia* makes them in some ways more valuable as objects of study than the previously mentioned cases. In fact such cells as these oöcytes might conveniently be used as tests for comparing the efficiency of various histological techniques. It is quite practicable to observe and to measure the elements of the nuclear complex in life, and then to recognise the same cell after fixation and mounting, and make comparative observations. The moderate-sized chromosomes have a length of about 10 μ hence they are sufficiently large to allow this.

SUMMARY.

1. The nuceolus of the resting oocyte represents a condensed chromatic spireme, hence it is identical with the total chromosomal contents of the nucleus.

2. During the early growth phases of the oöcytes, the nucleolus elongates and fragments. Each fragment has been identified as a pair of homologous chromomsomes indistinguishable united: later each of these bivalent elements divides in half, and the individual chromsomas: are thus separated. The two components of the largest bivalent element are unequal in size, and probably represent an XY pair.

3. The chromosomes can be counted either at the bivalent or at the univalent phase, the numbers obtained being 17 and 34 respectively.

4. At a still later stage the chromosomes fragment into numerous small globules, which become evenly distributed over the nucleus.

5. The whole of the account is based on observations made on living oocytes.

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EXPLANATION OF FIGURES.

All the figures, except fig. 23 were drawn from life with the aid of a Leitz Zeichen-Okular. Figures 2 to 22 were all drawn at the same magnification, using a 1/6 inch objective: the larger eggs hoever were subjected to a certain variable amount of pressure under the coverslip, hence the measurements are not in all cases strictly comparable.

Fig 1. A complete ovary, semi-diagrammatic.
Fig.3. - 12 are drawings of oöcytes.
Figs. 2 and 12 - 23 are oöcyte nuclei.
Fig. 23 A spermatocyte nucleus, in section.

For further descriptions see text.

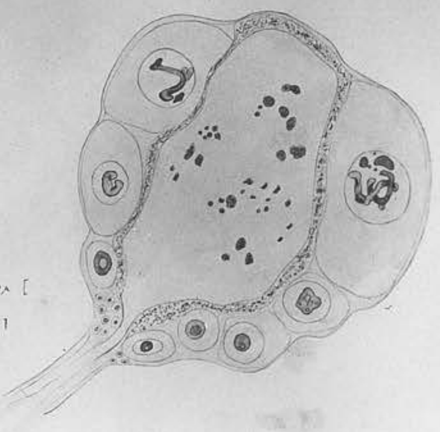

Fig 1

Fig 2

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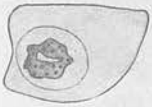

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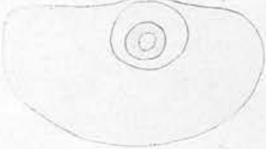

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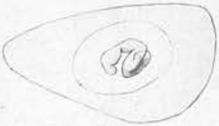

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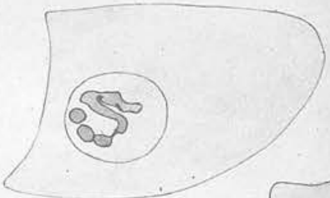

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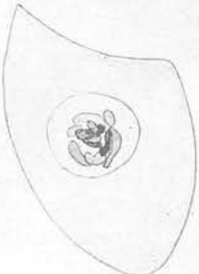

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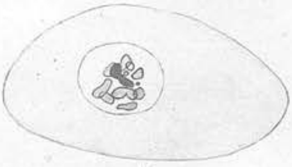
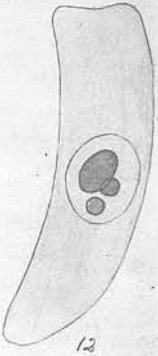


11 a



12

10 μ

13

14 a

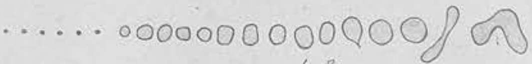
15 a

16 a

14 b.

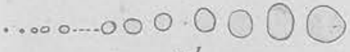
10 μ.

15 b.



16 b.

17 a



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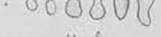

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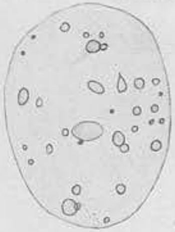

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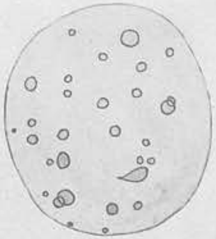
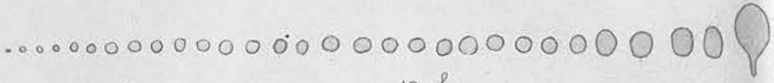


19 a

10 μ

18 b



19 b

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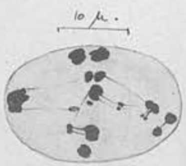

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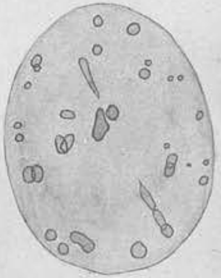

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PLATE 1.

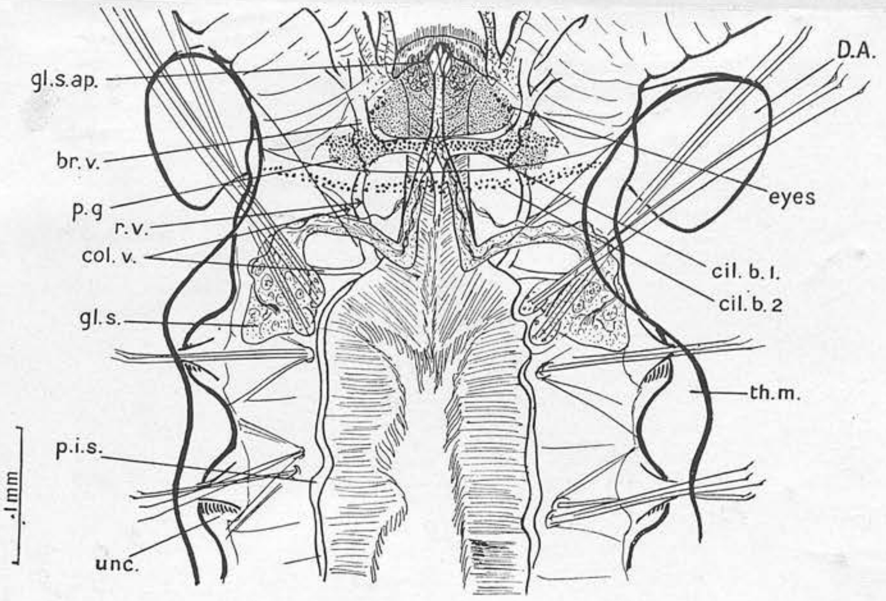


FIG. 1

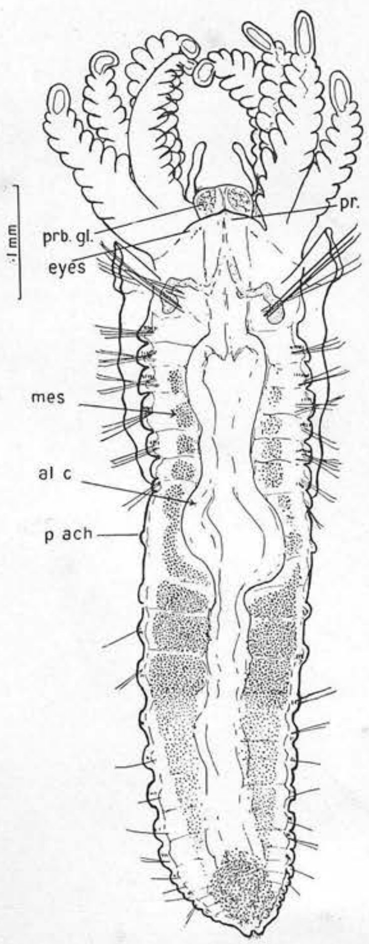


FIG 3

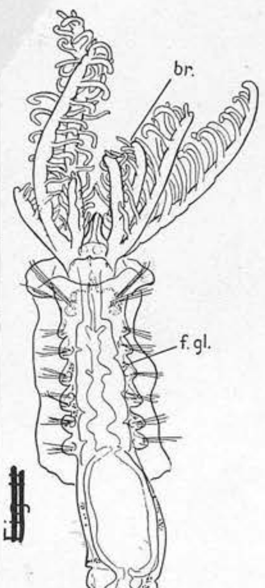


FIG 4

PLATE 2 (Figure 2).

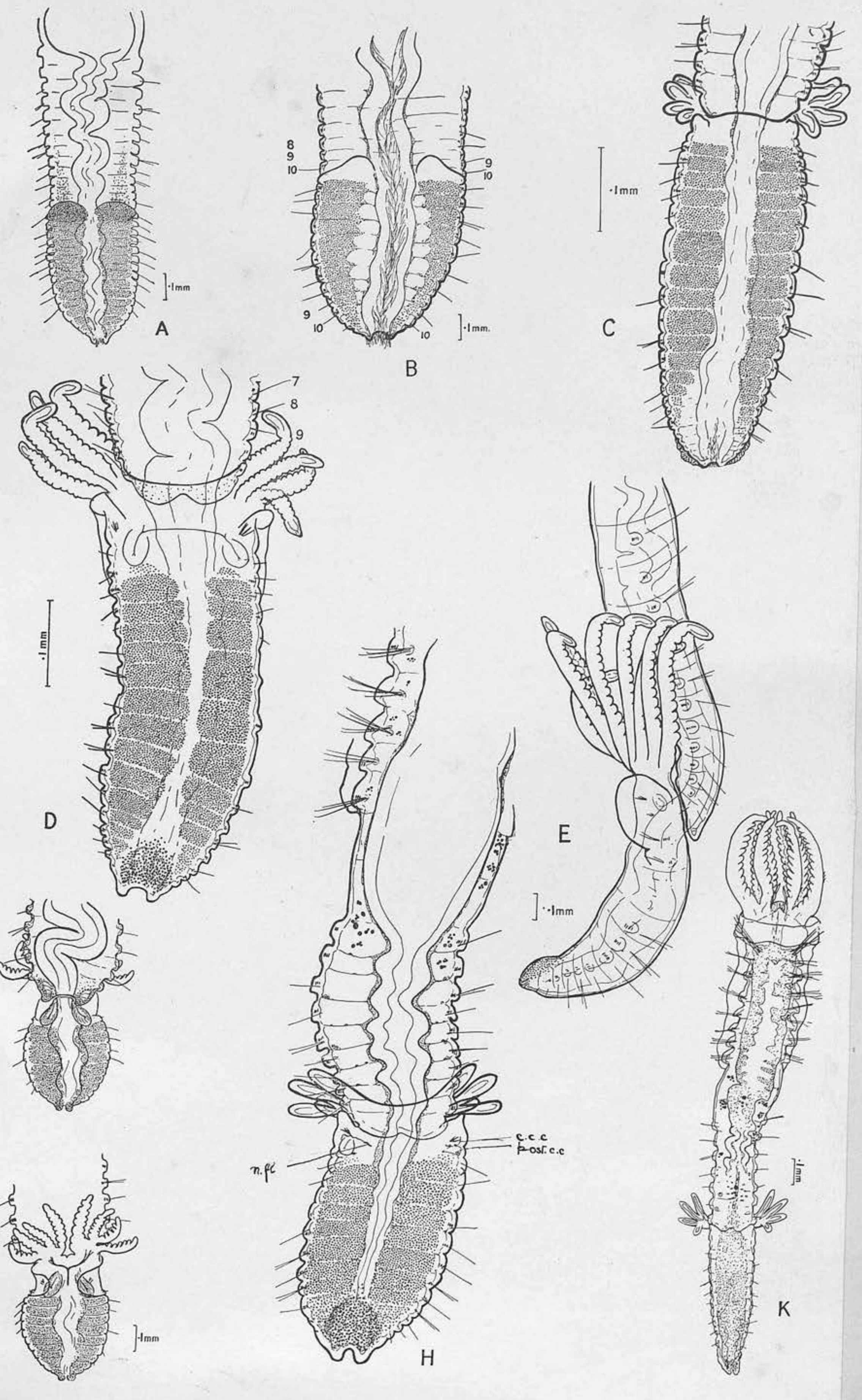


PLATE 3.

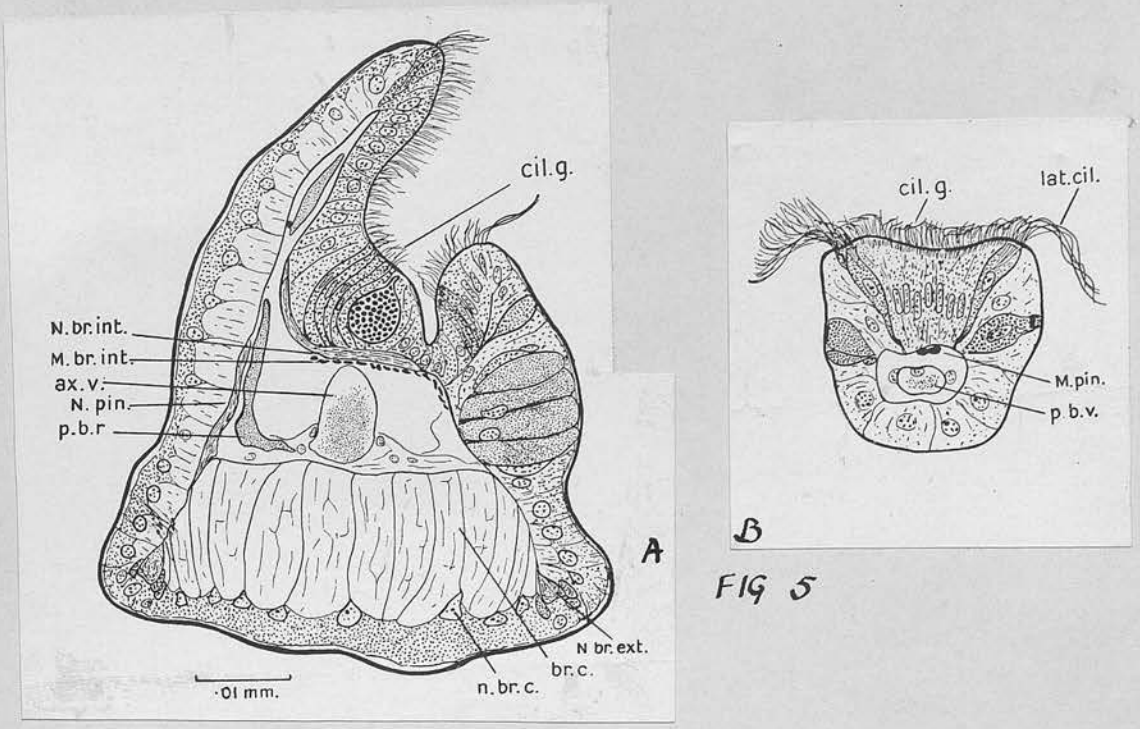


FIG 5

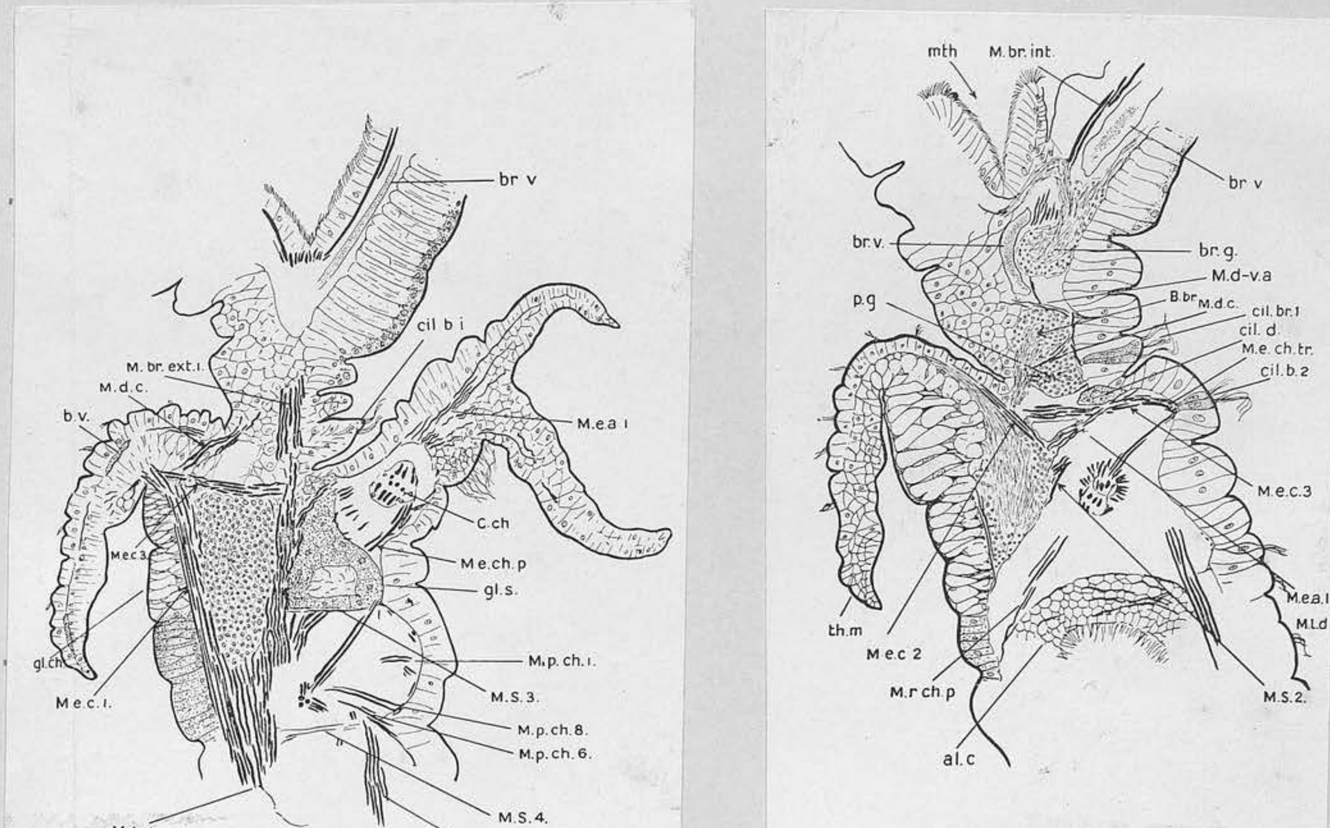


FIG 6

PLATE 4.

FIG 7

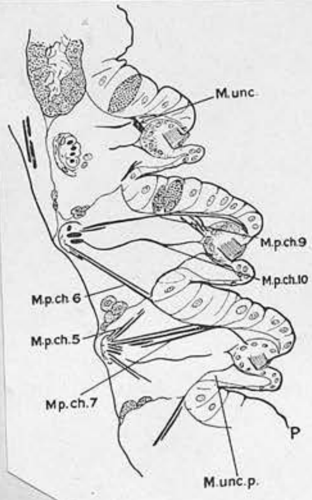


Fig. 8.

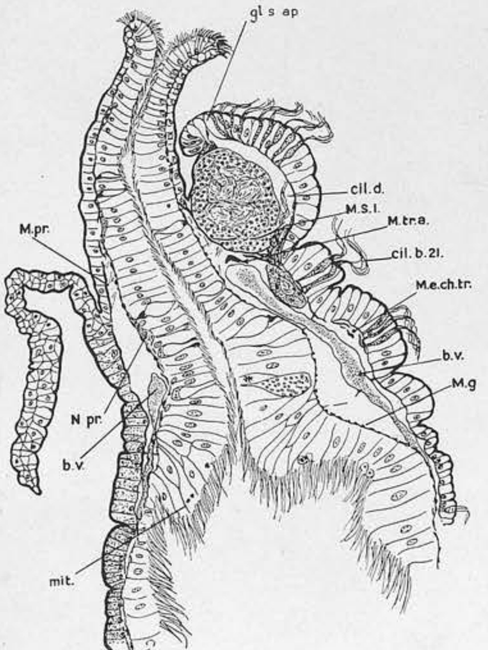


Fig. 9.

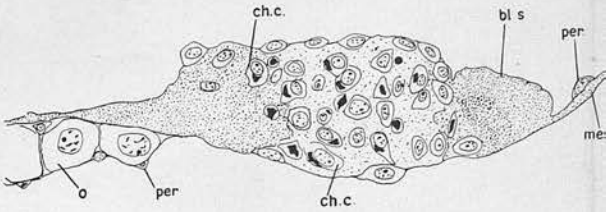
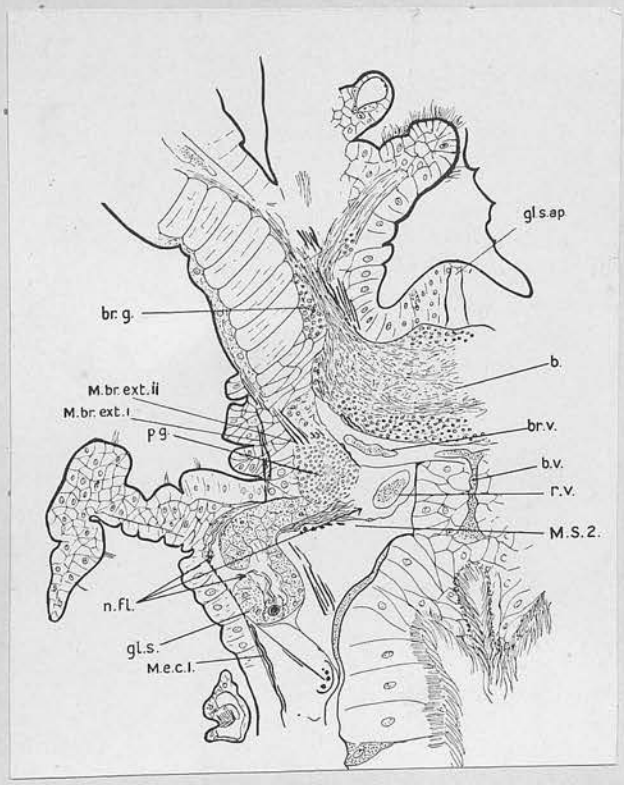


Fig. 10.

PLATE 5.



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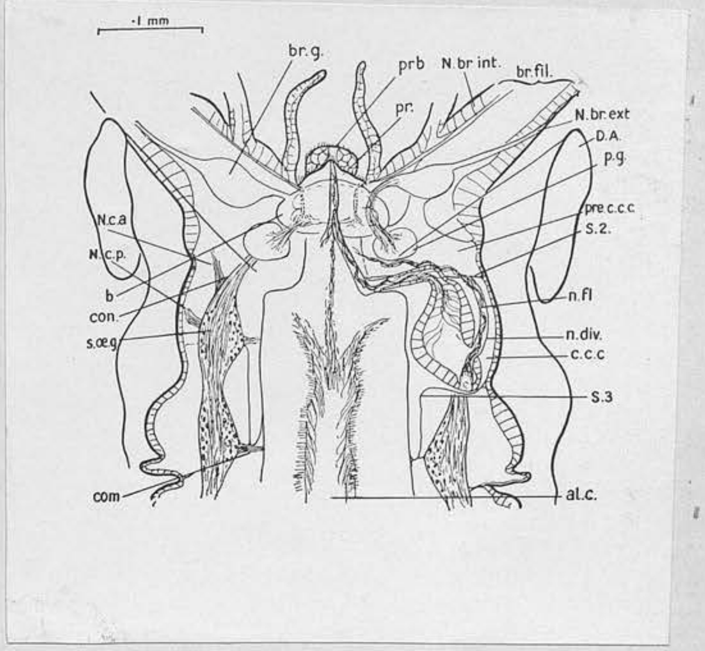


FIG 12

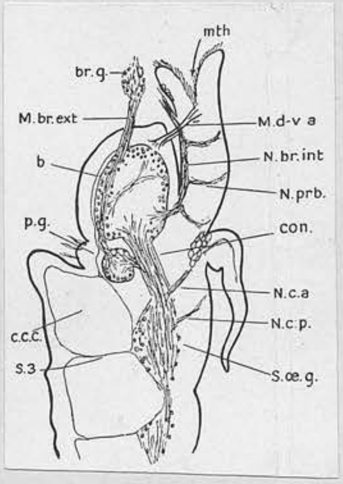


FIG 13

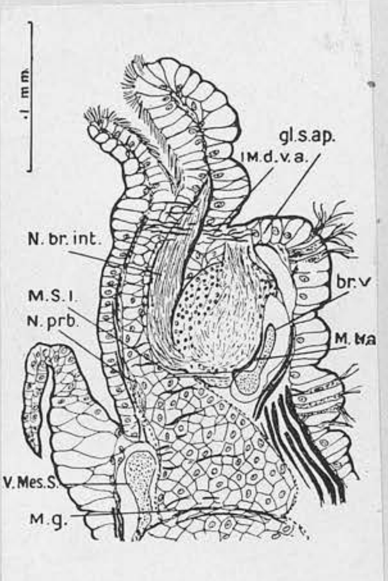


FIG 14

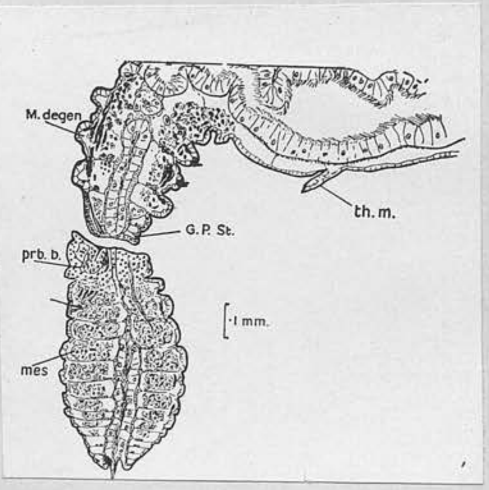


FIG 15

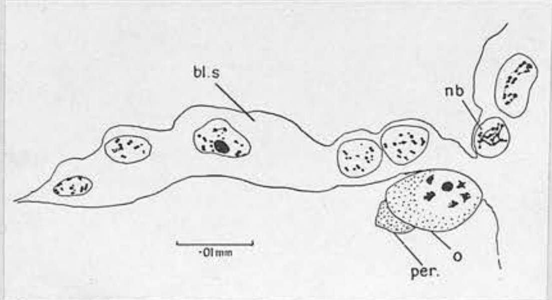


FIG 20

PLATE 6.

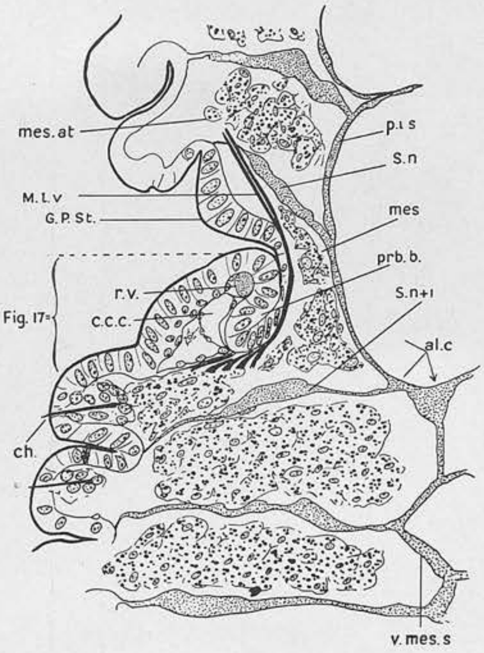


Fig. 16

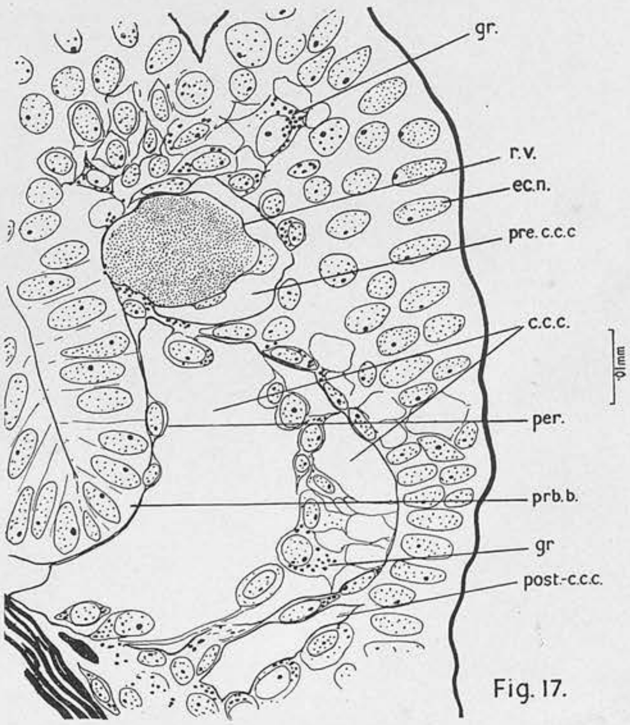


Fig. 17.

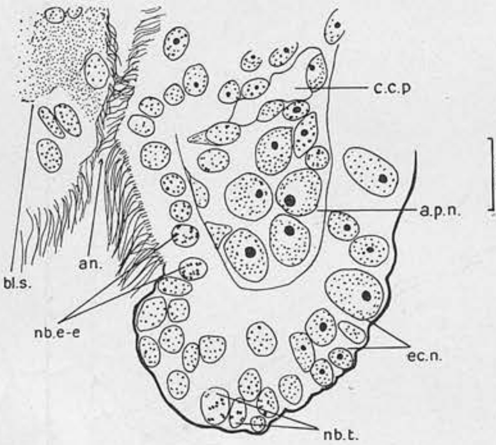


Fig. 19.

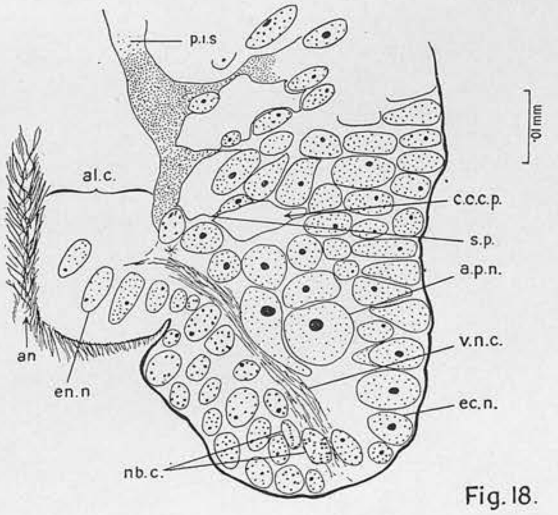
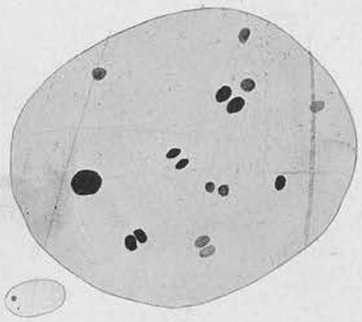


Fig. 18.

PLATE 7.



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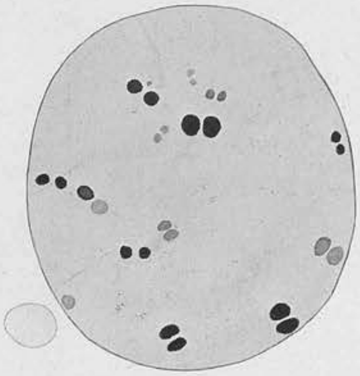
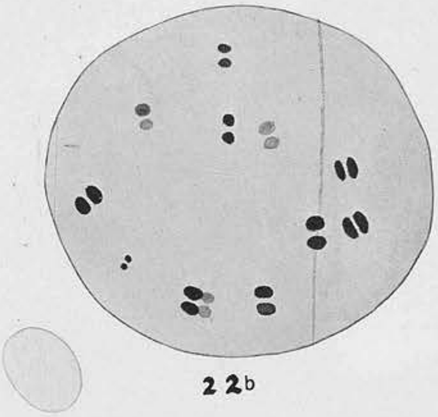


22a



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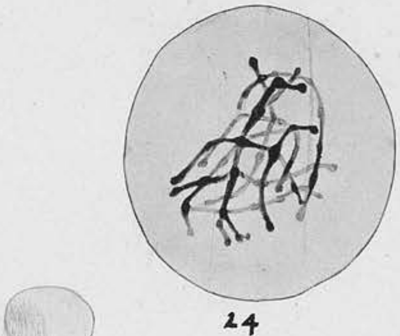

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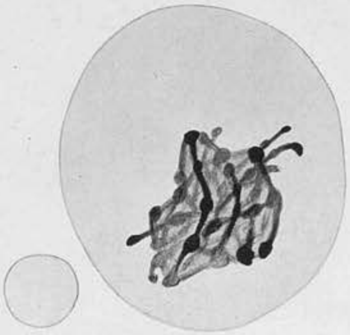
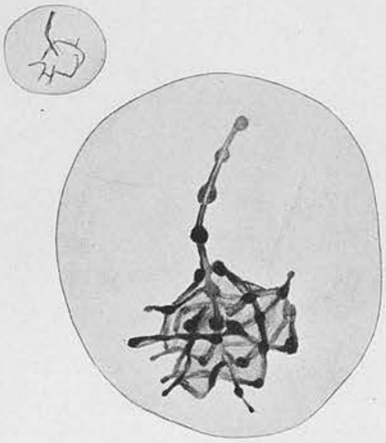


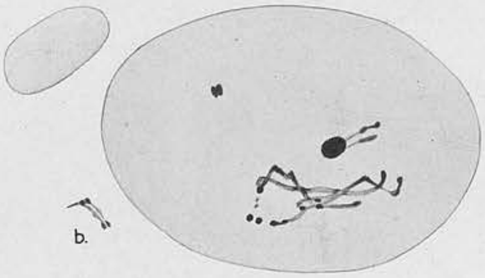
25a

·01 mm

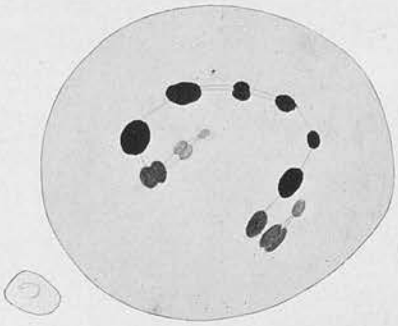
PLATE 8.



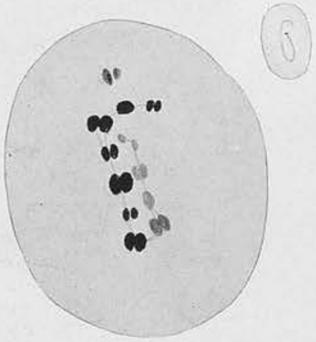
25 b



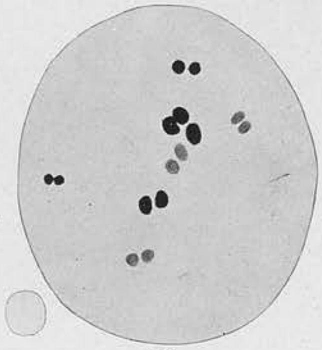
26 a & b



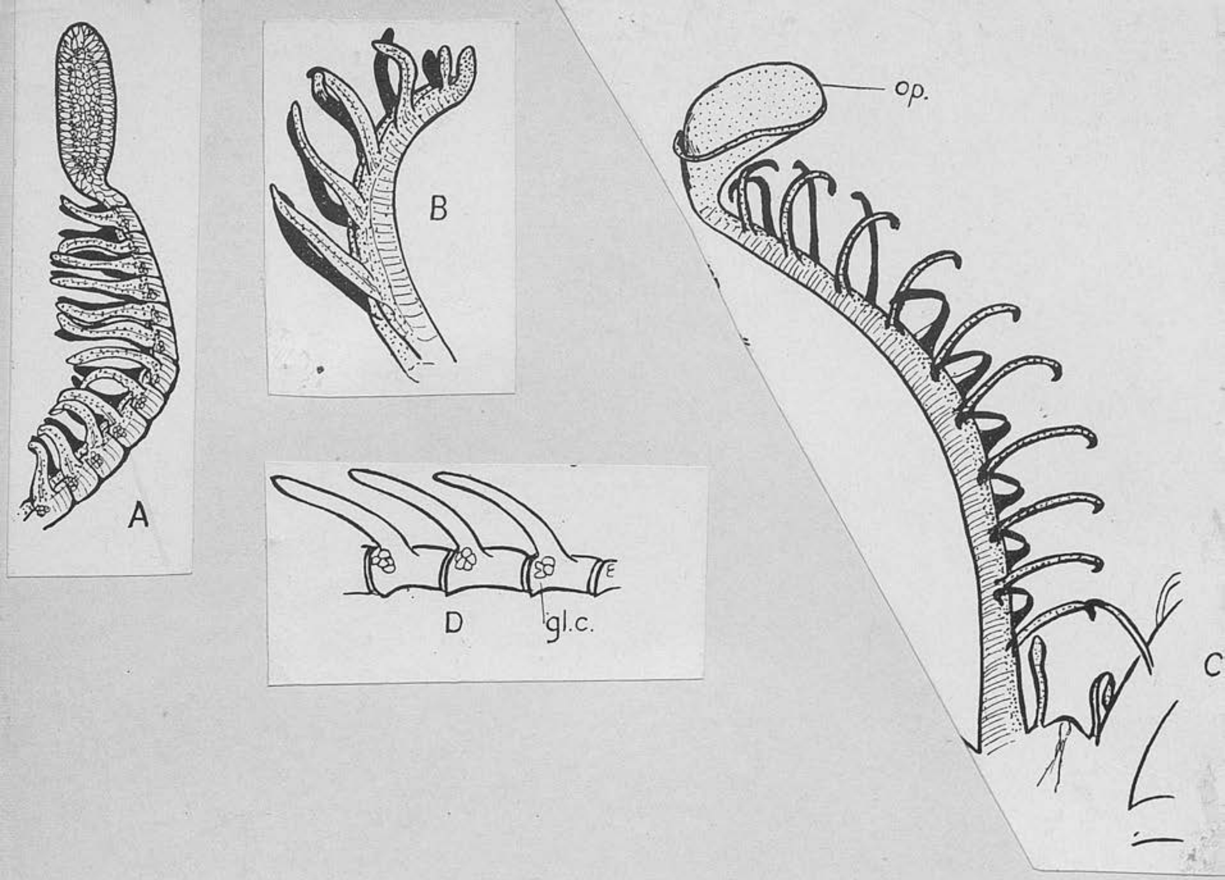
27 b



27 a



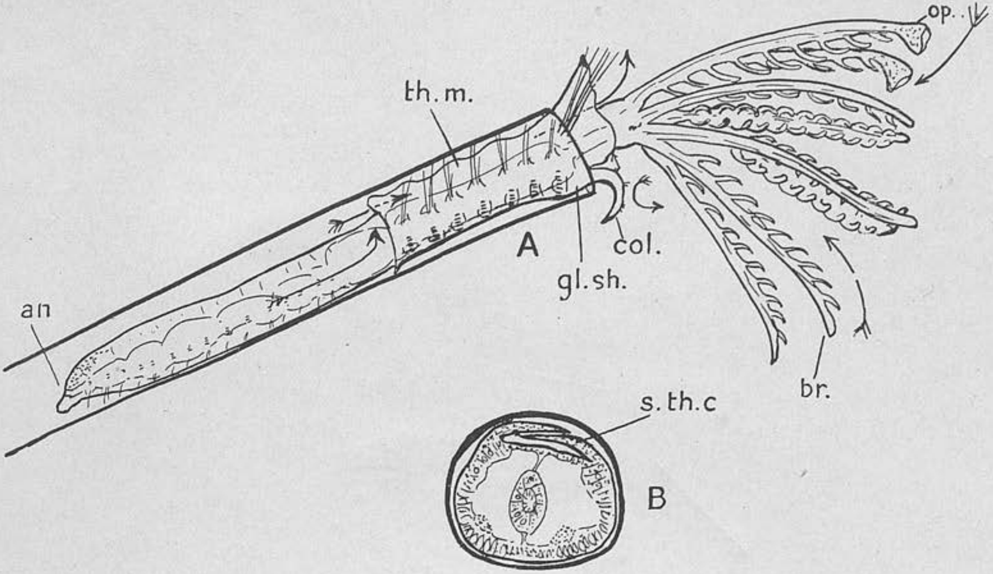
28



TEXT FIGURE 1. Branchial filaments of four individuals, showing variations of form.

- A. Filament with enlarged glandular termination.
- B. Terminal portion of filament without glandular enlargement.
- C. Dorsal filament of the operculate type.
- D. Three pinnules, with the group of glandular cells at their base.

gl.c. glandular cells: op. operculum.

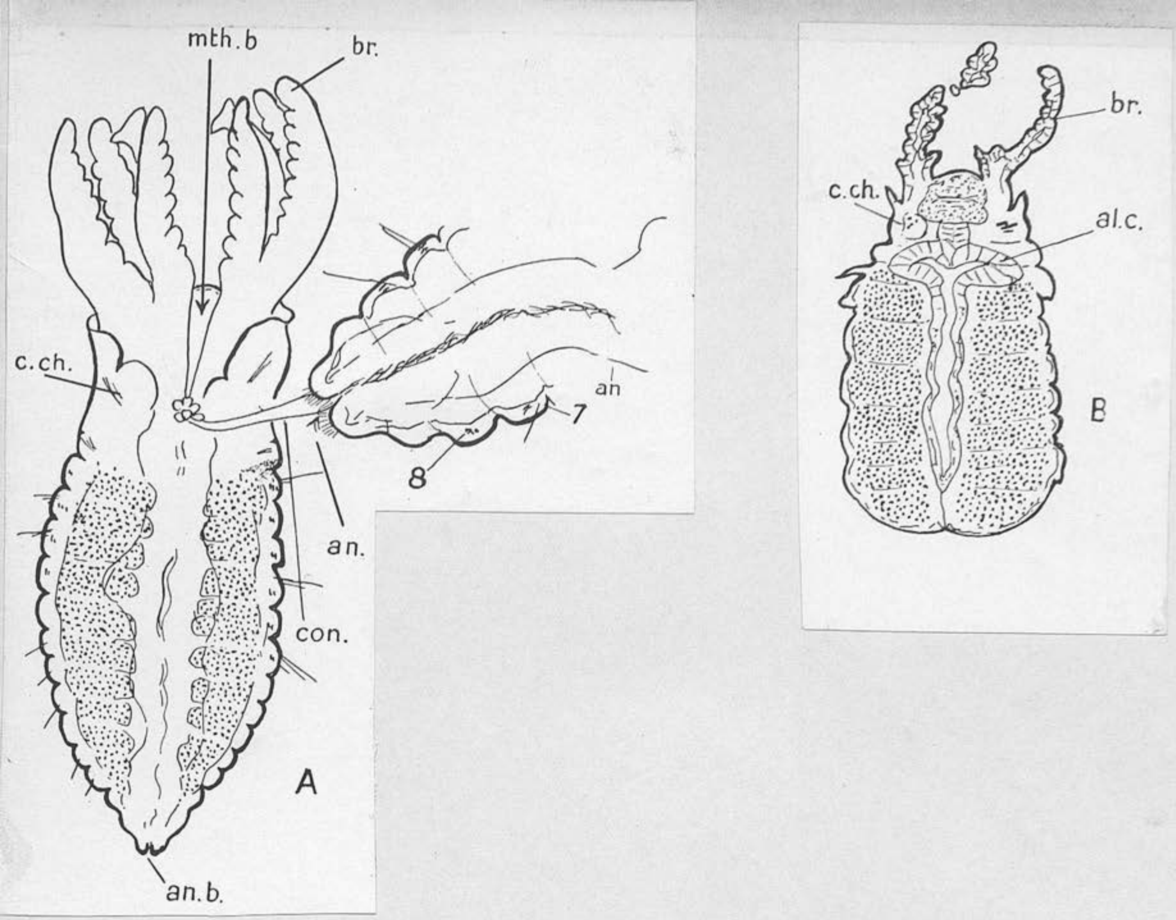


TEXT FIGURE 2.

A. The animal in its tube with the branchiae expanded: the arrows indicate the direction of the ciliary cerrents.

B. T.S. through the thoracic region of the animal in its tube.

an.	anus
br.	branchiae
col.	collar
gl.sh.	gland shield
op.	operculum
s.th.c.	supra-thoracic canal
th.m.	thoracic membrane.

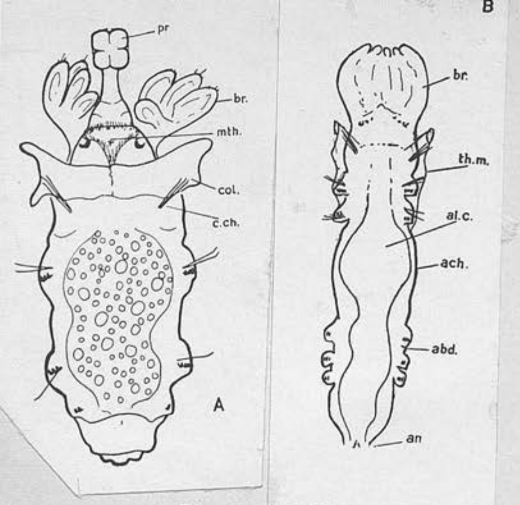


TEXT FIGURE 3.

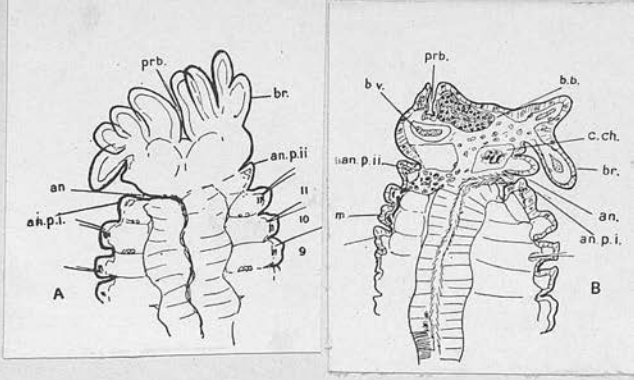
A. Bud and hind end of stock, at the time of separation of the bud (ventral view).

B. H.S. through a~~n~~ ~~almost fully developed~~ recently freed bud, to show the characteristic T-shaped gut.

al.c.	alimentary canal	an.	anus
an.b.	anus of bud	br.	branchiae
c.ch.	collar chaetae	mth.b.	mouth of bud
con.	connecting filament between bud and stock.		



Text fig 4



Text fig 5

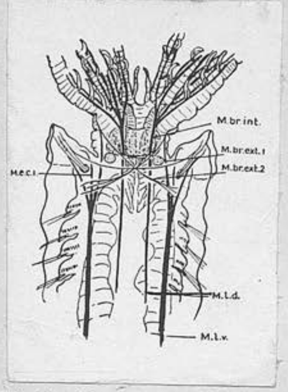
TEXT FIGURE 4.
A. Larva immediately after settling (length 700).
B. Larva one month after settling.

abd.	abdomen	ach.	achaetous zone
al.c.	alimentary canal	an.	anus
br.	branchiae	col.	collar
c.ch.	collar chaetae	mth.	mouth
pr.	prostomium	th.m.	thoracic membrane.

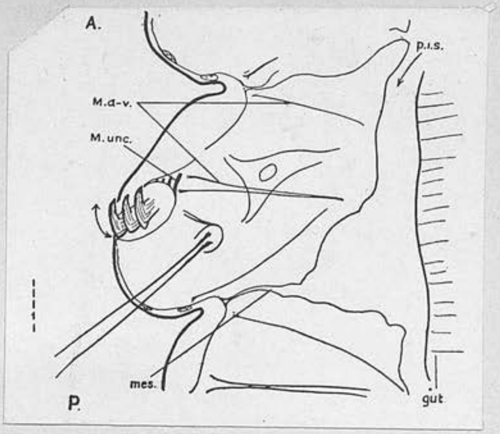
TEXT FIGURE 5. Abnormal terminal bud.
A. Sketch of external appearance of bud head.
B. Horizontal section through same region.

al.c.	alimentary canal	an.	anus.
an.p.i., & an.p.ii.,	anal papillae of stock		
b.b.	brain of bud	br.	branchiae of bud
c.ch.	collar chaetae of bud	m.	muscles of stock
prb.	proboscis of bud	b.v.	blood vessel.

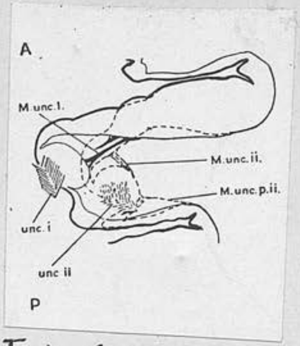
The figures indicate the numbers of the segments as counted from the anterior end of ebdomen of stock.



Text fig 6



Text fig 7



Text fig 8

TEXT FIGURE 6.

Diagramatic dorsal view of anterior end to show musculature of head and branchiae.

M. br. int.	Internal branchial muscle
M.br.ext. 1&2	External branchial muscles
M.e.c.l.	Erector muscle of collar
M.e.ch.p.	Posterior erector of collar chaetae
M.l.d.	Dorsal longitudinal muscle
M.l.v.	Ventral longitudinal muscle.

TEXT FIGURE 7.

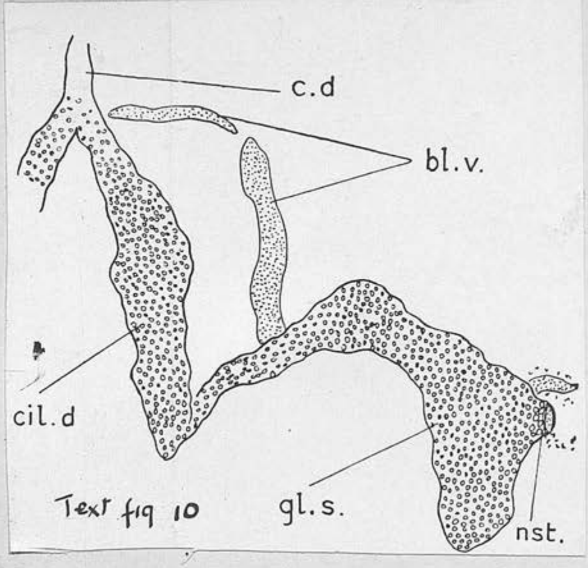
Horizontal view of abdominal parapodium, as seen in the living animal. A&P indicate anterior and posterior.

M.d-v	Dorso-ventral muscles:	M.unc.	uncinal muscles
mes.	mesentery	p.i.s.	peri-intestinal sinus.

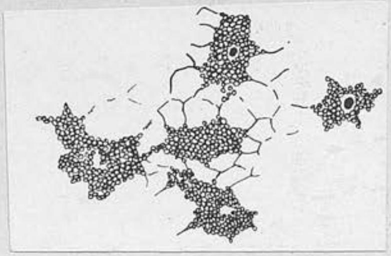
TEXT FIGURE 8.

Camera lucida drawings of two adjacent thoracic parapodia superimposed: for explanation see p.80. A & P indicate anterior and posterior.

unc.i. uncinus of first somite: M.unc.i. corresponding muscle.
unc.ii. uncinus of second somite:
M.unc.ii, corresponding muscle (contracted)
M.unc.p.ii. accessory retractor muscle of uncini of second somite (contracted).



Text fig 10

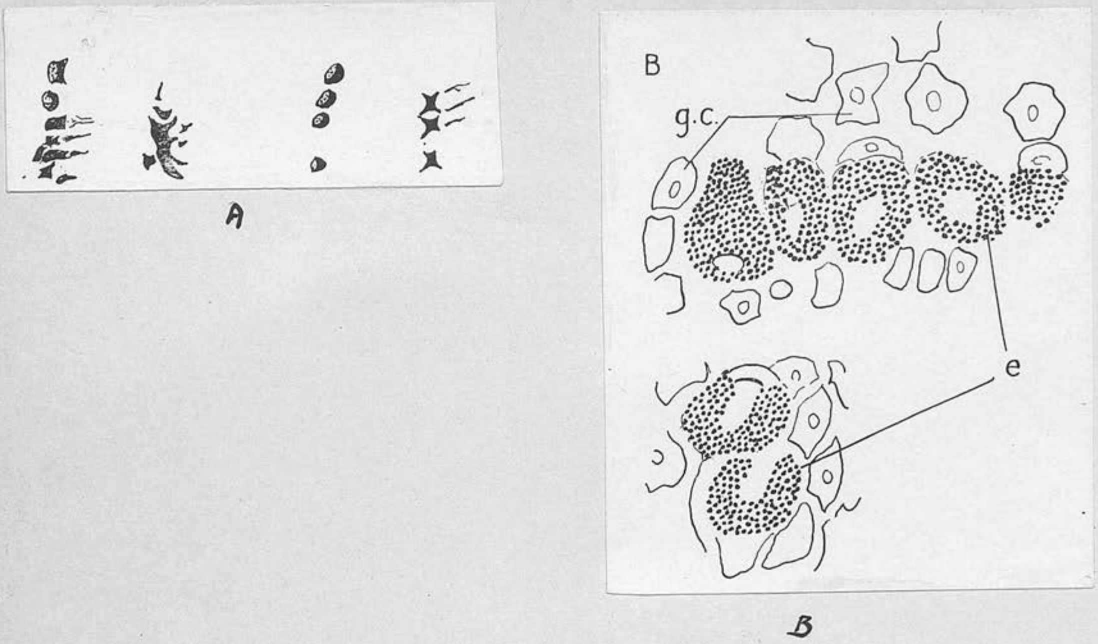


Text fig 11

TEXT FIGURE 10.
One of the two glandular sacs, as seen in life in a particularly favourable individual, showing the nephrostomial opening.

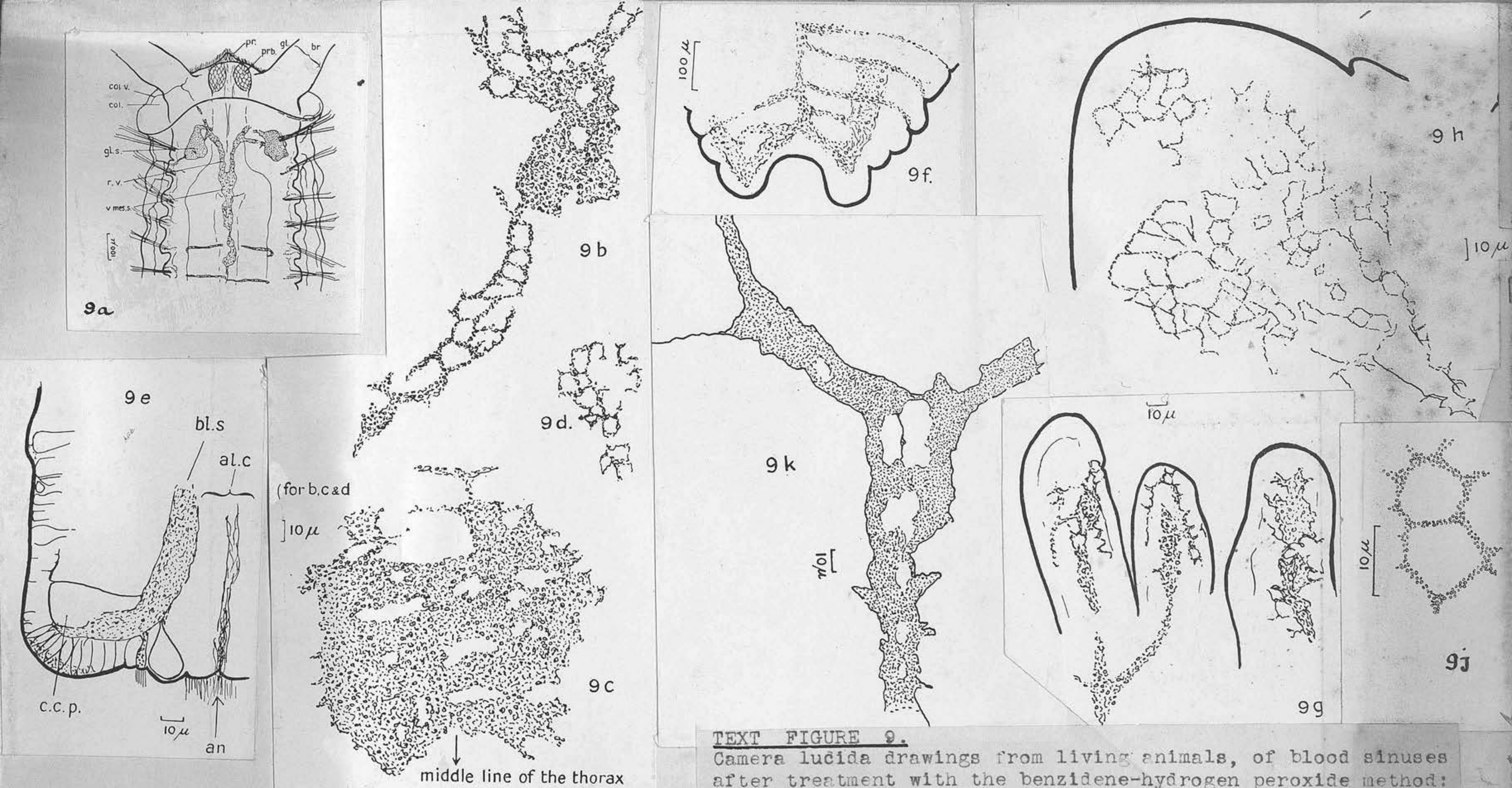
bl.v.	blood vessel	c.d.	common duct of the two sacs.
cil.d.	ciliated duct		
gl.s.	glandular sac	nst.	nephrostome.

TEXT FIGURE 11.
Excretory cells in ventral body wall - surface view - as seen in life after treatment with methylene blue.



TEXT FIGURE 12.
A. External appearance of the row of eyes of four individuals.
B. The eyes as seen in transverse section.

e. eye - the pigmented cup.
g.c. ganglion cells of the brain.



TEXT FIGURE 9.
Camera lucida drawings from living animals, of blood sinuses after treatment with the benzidene-hydrogen peroxide method: for explanation see text page 101 et seq..

a. Ventral view of thorax.
b. Portion of vantral vessel in thorax.
c. Portion of mid-dorsal body wall, immediately posterior to hind end of proboscis.
d. Honeycomb of small size, in ventral edge of collar.
e. Anal region, to show the termination of the peri-enteric sinus (from an unstained animal)
f. The same region as e, stained with benzidene.
g. Branchiae of bud showing superficial (sub-epidermal)network in continuity with the branchial vessel.
h. A specially good honeycomb on the branchiae of a bud (under a 1/6inch objective).
j. A few cells of the same, drawn under a 1/16 oil immersion lens.
k. Bifurcation of dorsal vessel of bud.

al.c.	alimentary canal
br.	branchiae
bl.	blood sinus
c.c.p.	posterior coelimic cavity
col.	collar
gl.s.	glandular sac
pr.	prostomium
prb.gl.	proboscis gland
col.v.	collar vessel
r.v.	ring vessel
v.mes.s.	ventral mesenteric sinus.